

name	symbol	Applied Bioystems "assay on demand" assay #	forward primer	Seq ID No.	reverse primer	Seq ID No.	Seq ID No.
aspirin (ir class 1)	ASPN		AATACAAAGGACACATCAAGGA	1	TCTTCTCTCAATTCGATATGGA	23	TTGGAAATGAGTGCAAAAGCTCTCTGATTAATG
chondroitin sulfates proteoglycan 2 (version)	CSPG2		GCCAGTGGATGATGTTCC	2	TCTTGGCAATTTCTACACAGGG	24	AGGCAAGATTTGCTTGGGCGAGC
cystatins SN, SA & S	CST1, 2, 4		AGTCCAGGCCACTCTGGA	3	GGGAAGCTTGTGATGCTGGAAAGA	25	AGGCAAGATTTGCTTGGGCGAGC
gamma-glutamyl hydrolase	GGH		GTGGCCATGGCGCTGAA	4	TGACAGCAACACTCTAGTAGGAAA	26	TTGCTGGAGTTCATTTGCCACAGCAGAT
heparin-like growth factor binding protein 7	IGFBP7		CAGGTGACAGAGGCGACC	5	TGACAGCAACACTCTAGTAGGAAA	27	AGGCAAGATTTGCTTGGGCGAGC
heparin 10	KLK10		ACACATGATGTTGCTGGAGTGG	6	GAGAGGATGCTTGGAGGTT	28	CTTCCAGAGTGAATCTGGAGGCGC
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1		CTTGAGTACAGGCTGAGCTTTC	7	CGGTGACACAGTCTCTTTACAG	29	CGATCAGAGTCACTACATTCAGGTTCTCA
lumican	LUM		GATCTCTGTGCATGTCATCTGC	8	CCATCAATGCCAGGAGAGA	30	TAGGATTCAAAGCATTTGGCAAAAATGAGTCTAA
lyso oxidase-like 2	LOXL2		AGGCCAGCTCTCTGCTTGG	9	CCCTGATGCGCGAGTTG	31	CGTAACTCTCTGATGCTCTCTCTCAATCTG
matrix metalloproteinase 12	MMP12		GGCTCTCTGCTGATGATAGT	10	AGTGACAGCATCAAAATCTCAATTG	32	TCAGTCTCTGATGAGAGCGCCAAAGAGAA
metalloproteinase inhibitor 1	THP1		CCGAGCACCTTATACACGG	11	GGAGCTGTGGAGTATGCGC	33	CAAGATGACCAAGATGATATAAGGGTTTCAAGC
n-acylsphingosine amidohydrolase	ASAH1		GGCAGAACGCTGCGAAA	12	ACAGGACATCATCATGTTTCAAA	34	TGCTTGACCGGACCGACCGCAGAGAGA
secreted frizzled-related protein 2	SFRP2		GGCTAGCAGGAGCAAGCT	13	TTTGGCAGGCTTCATACATCTTT	35	CTGCGAGCGACCGAGGAGAGCTC
secreted protein, acidic, cysteine rich	SPARC		TTCTCCGTGACAGTGGCAGTTC	14	GAAAAGCGGGTGGTGA	36	TTGGACCGAGCGCGCATTTGAGGG
serine protease 11 (IGF binding)	PRSS11		TGGGAGCGCGGTTAGTAA	15	AAGGAGTTCCAGCTCTCTCTTTC	37	AGTGTAAITTCATCACTCTCCAGGTTCAAGG
thrombospondin 2	THBS2		TGGAGGAGTACAGCGGCTATAG	16	TAGGTTTGTCATAGTAGGTTCTGAGT	38	AGGCGCAGAGCGGCTACATCAGATC
thyroglobulin	TG		GAGGTTCTCTGCGAGTTCAA	17	TGTAAAGCGCTCCATCTTCAT	39	TGTGGCAGATTCGATTCGCGCAA
human cell growth regulator with EF hand domain 1	GR11		CTGCGCACCGCTTCCA	18	TTCTGTCTCTCTAGTCTTTTAGG	40	CGAGCGAGGAGGAGGCTGG
human serine or cysteine proteinase inhibitor clade B transforming growth factor beta	SERPINE5		TCGACGCAATTTCCAGGATA	19	AAGCGCAATTTCTAGTATGGA	41	TCAGTCCAGCGCGCGCAATGGA
transforming growth factor beta	TGFB1		GGTCCATGTCATCCACATGTT	20	TCTGCAAGTTCATCCCTCTTT	42	CAAGCTCCAGCGCACAGAGCTCAGG
human proprotein convertase subtilisin/kexin type 5	PCSK5		AAAATCTTTCGCGGAAATGC	21	JAGTCTGGCGGTTGAAATAC	43	ACAGAAATGAGGATGGGTTAGCGTGA
matrix metalloproteinase 2	MMP2		TTGATGCTGCTGCTCAGATC	22	TGTCAGTGGCGTCAAGT	44	TTCAAGGACCGGTTCAATTTGGGG
human serine or cysteine proteinase inhibitor clade H adiclin	SERPINE1	Hs00241844 m1 Hs00377849 m1					
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	Hs00213545 m1					
secreted frizzled-related protein 4	SFRP4	Hs0180066 m1					
Inhibin beta A chain	INHBA	Hs00170103 m1					
osteopontin	OPN1	Hs00167093 m1					
transforming growth factor beta-induced	TGFB1	Hs00165908 m1					

Figure 1

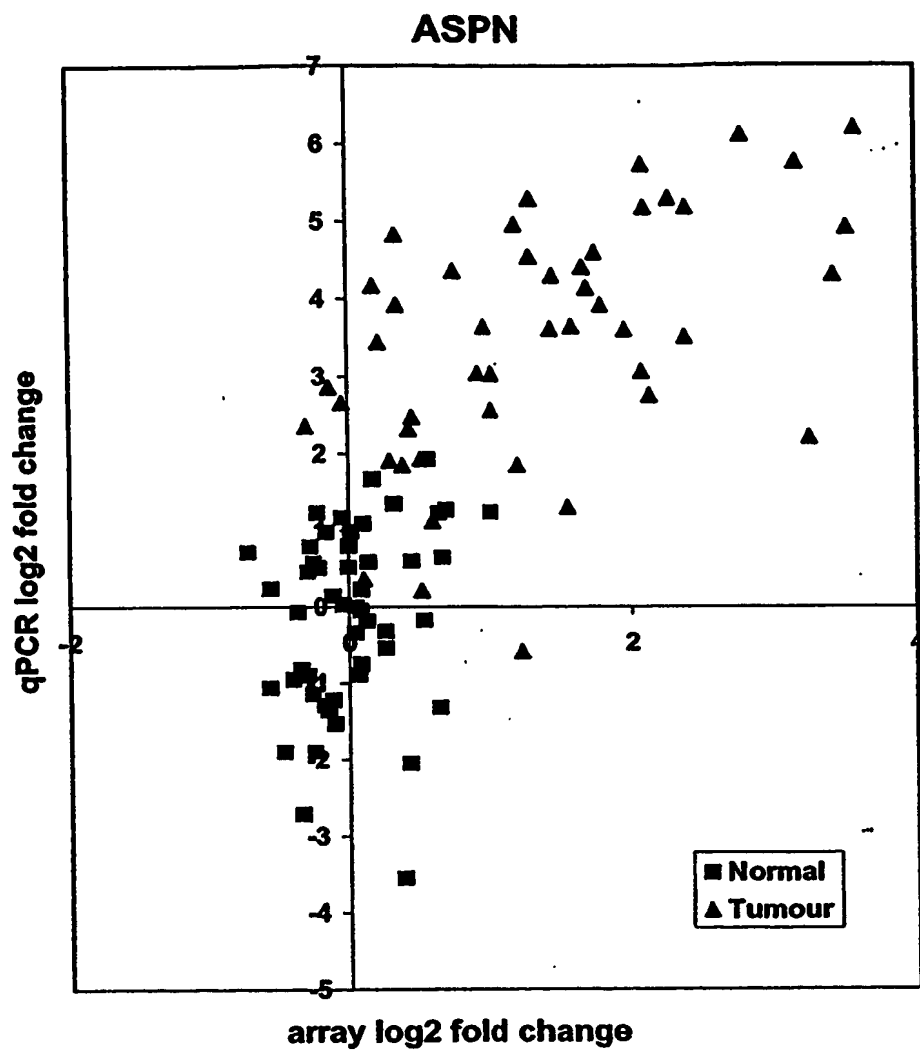
Abstract - Identification of Markers for Gastric Malignancy

Microarray - Identification of Markers for Gastric Malignancy													
name	symbol	MWG oligo #	NCBI		fold change	fold change rank	original t-test	Bonferroni-adjusted p value	2 sample Wilcoxon test				
			mRNA ref sequence	protein ref sequence									
-	-	C:0531	NM_015419	NP_060150	1.8	-17818	1.0E-28	3.04E-24	0.0E+00				
adiponin (rr class 1)	ASPN	A:07749	NM_017680	NP_060150	2.6	-22292	6.4E-23	1.9E-18	0.0E+00				
carboxypeptidase N	CPN2	B:4922	-	P22792	2.7	-22367.5	2.3E-42	7.0E-38	0.0E+00				
cell growth regulatory factor with EF-hand domain	GRL1	A:07876	NM_006569	NP_006560	3.0	-21188.5	4.33E-42	1.3E-37	0.0E+00				
chondroitin sulfate proteoglycan 2 (versican)	CSG2	A:10008	NM_004385	NP_004376	2.3	-21606.5	2.23E-33	6.65E-29	0.00E+00				
cystatin SN	CST1	A:06089	NM_001889	NP_001889	2.1	-17475	1.3E-18	3.8E-14	0.0E+00				
cystatin SA	CST2	A:06089	NM_001322	NP_001313	2.1	-17475	1.3E-18	3.8E-14	0.0E+00				
cystatin S	CST4	A:06089	NM_001899	NP_001890	2.1	-17475	1.3E-18	3.8E-14	0.0E+00				
eef-containing fibulin-like extracellular matrix protein 2	EFPMP2	A:09072	NM_016938	NP_058634	2.4	-22761	2.0E-35	5.9E-31	0.0E+00				
gamma-glutamyl hydrolase	GGH	A:03601	NM_003878	NP_003869	1.6	-18092	1.6E-07	4.8E-03	5.7E-11				
inhibin beta A chain	INHBA	A:02189	NM_002192	NP_002183	2.1	-21247	1.4E-30	4.3E-26	0.0E+00				
insulin-like growth factor binding protein 7	IIGFBP7	A:03385	NM_001553	NP_001544	3.0	-25854	5.4E-31	1.6E-26	0.0E+00				
kallikrein 10	KLK10	A:07907	NM_002776	NP_002767	2.3	-17986.5	5.0E-10	1.5E-05	4.9E-06				
leucine proline-enriched proteoglycan 1(leprecan 1)	LEPRE1	A:04646	NM_022356	NP_071751	1.7	-18019	8.2E-14	2.4E-09	1.1E-12				
lumican	LUM	A:09199	NM_002345	NP_002336	2.9	-24927	4.2E-24	1.3E-19	0.0E+00				
lysyl oxidase-like 2	LOXL2	A:06085	NM_002318	NP_002309	1.6	-16994.5	5.9E-10	1.7E-05	7.9E-10				
matrix metalloproteinase 2	MMP2	A:06749	NM_004530	NP_008253	1.8	-18710	1.2E-11	3.6E-07	1.5E-10				
matrix metalloproteinase 12	MMP12	A:01762	NM_002426	NP_002417	2.1	-20209.5	2.2E-12	6.6E-08	4.9E-11				
metalloproteinase inhibitor 1	TIMP1	A:08048	NM_003254	NP_003245	3.2	-24177	7.5E-38	2.3E-33	0.0E+00				
n-acetylglucosamine amidohydrolase	ASAHI	A:10030	NM_004315	NP_004306	1.7	-19636.5	9.6E-16	2.9E-11	0.0E+00				
olefactorin	OIFM1	B:3555	NM_014279	NP_055094	3.9	-25782.5	6.5E-46	1.9E-41	0.0E+00				
osteopontin	SPP1	A:09441	NM_000582	NP_000573	7.0	-26668	4.0E-32	1.2E-27	0.0E+00				
human proprotein convertase subtilisin/kexin type 5	PCSK5	A:00704	NM_006200	NP_006204	1.7	-18736	2.0E-11	6.0E-07	7.3E-11				
group xiii secreted phospholipase a2	PLA2G12b	B:1811	NM_032562	NP_115951	3.0	-23212	7.9E-39	2.36E-34	0.00E+00				
secreted frizzled-related protein 2	SFRP2	B:1634	XM_050625	XP_050625	2.1	-19217	2.7E-10	8.1E-06	4.1E-08				
secreted frizzled-related protein 4	SFRP4	A:07398	NM_003014	NP_003005	3.0	-22153	6.0E-24	1.8E-19	0.0E+00				
serine (or cysteine) proteinase inhibitor clade H	SERPINF1	A:06615	NM_001235	NP_001226	1.9	-20252	2.8E-34	8.2E-30	0.0E+00				
human serine or cysteine proteinase inhibitor clade B	SERPINB5	A:10485	NM_002639	NP_002639	1.5	-17026	4.6E-06	1.4E-01	5.6E-06				
serine protease 11 (IGF binding)	PRSS11	B:1274	NM_002775	NP_002766	1.6	-17184.5	9.3E-10	2.8E-13	0.0E+00				
secreted protein, acidic, cysteine rich	SPARC	A:08092	NM_003118	NP_003109	2.5	-22947.5	1.5E-44	4.6E-40	0.0E+00				
spandin 2	SPON2	B:2543	NM_012445	NP_036577	2.4	-20390.5	2.9E-31	8.5E-27	0.0E+00				
stannin	SNIN	A:09316	NM_003498	NP_003489	2.1	-20162.5	3.25E-24	9.71E-20	0.00E+00				
thrombospondin 2	THBS2	B:9017	NM_003247	NP_003238	2.6	-22095	5.8E-29	1.7E-24	0.0E+00				
thrombospondin repeat containing 1	TSRC1	B:7686	NM_019032	NP_061905	2.6	-22608	1.39E-45	4.1E-41	0.0E+00				
thyroglobulin	TG	B:5402	NM_003235	NP_003226	2.4	-23644	4.39E-36	1.3E-31	0.0E+00				
transforming growth factor beta-induced	TGFB1	A:08124	NM_000358	NP_000349	2.5	-23339.5	1.96E-24	9.71E-20	0.0E+00				
transforming growth factor beta1	TGFB1	A:07050	NM_000660	NP_01137	1.6	-17214	2.30E-18	6.86E-14	0.0E+00				
hyaluronan and proteoglycan link protein 4	HAPLN4	C:6300	NM_023002	NP_075378	3.4	-23516.5	7.32E-44	2.2E-39	0.0E+00				

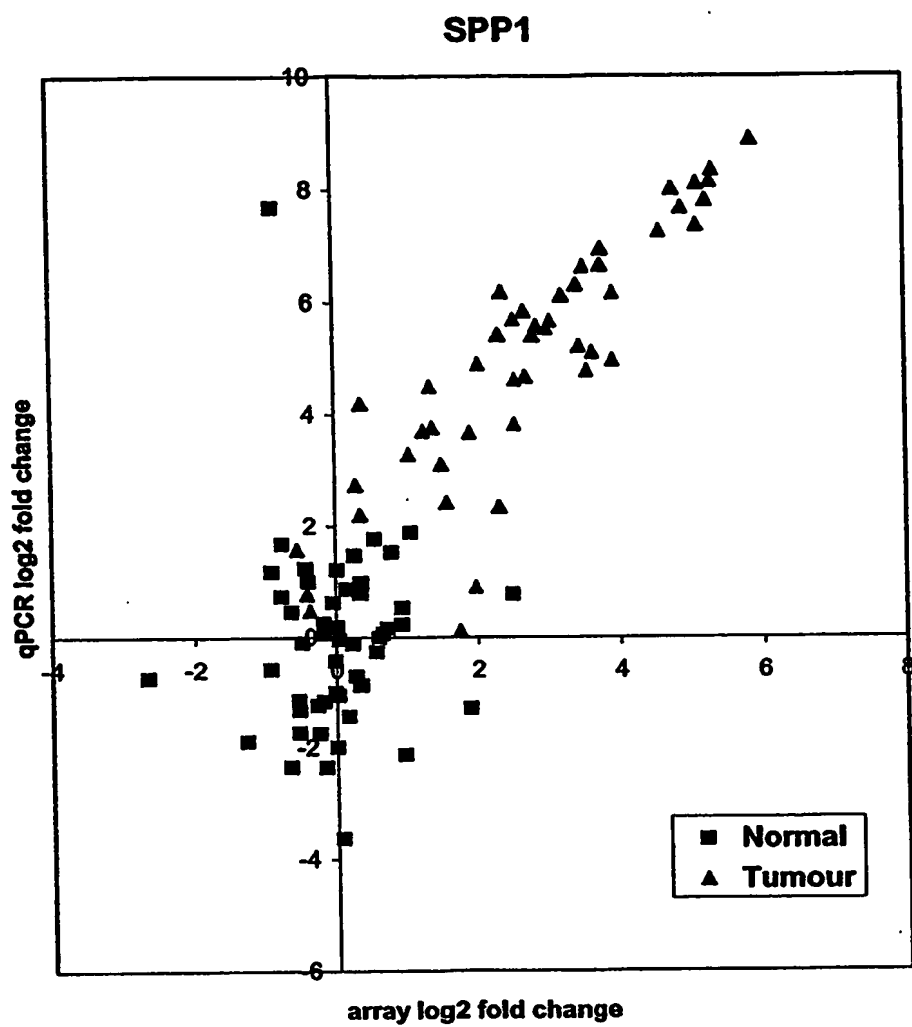
Figure 2

Quantitative RT-PCR - Quantification of Expression of Selected Gastric Cancer Candidate Genes				
name	symbol	median T:N fold change	Maximum T:N fold change	% T >95th percentile
adipican		5	37	74
asporin (Irr class 1)	ASPN	12	73	91
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	6	24	78
cystatins SN, SA & S	CST1, 2, 4	525	25532	100
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	3	15	56
gamma-glutamyl hydrolase	GGH	5	36	67
inhibin beta A chain	INHBA	34	357	98
insulin-like growth factor binding protein 7	IGFBP7	4	19	80
kalikrein 10	KLK10	5	633	70
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1	4	17	72
lumican	LUM	5	47	80
lysyl oxidase-like 2	LOXL2	6	26	93
matrix metalloproteinase 12	MMP12	9	586	67
metalloproteinase inhibitor 1	TIMP1	8	19	91
n-acylsphingosine amidohydrolase	ASAH1	3	7	63
osteopontin	SPP1	40	481	96
secreted frizzled-related protein 2	SFRP2	5	85	63
secreted frizzled-related protein 4	SFRP4	56	600	100
secreted protein, acidic, cysteine rich	SPARC	9	56	93
serine protease 11 (IGF binding)	PRSS11	4	25	54
thrombospondin 2	THBS2	25	239	91
thryoglobulin	TG	5	153	54
transforming growth factor B-induced	TGFBI	7	204	82
1 percentage of tumors with expression levels greater than the 95th percentile of non-malignant samples.				

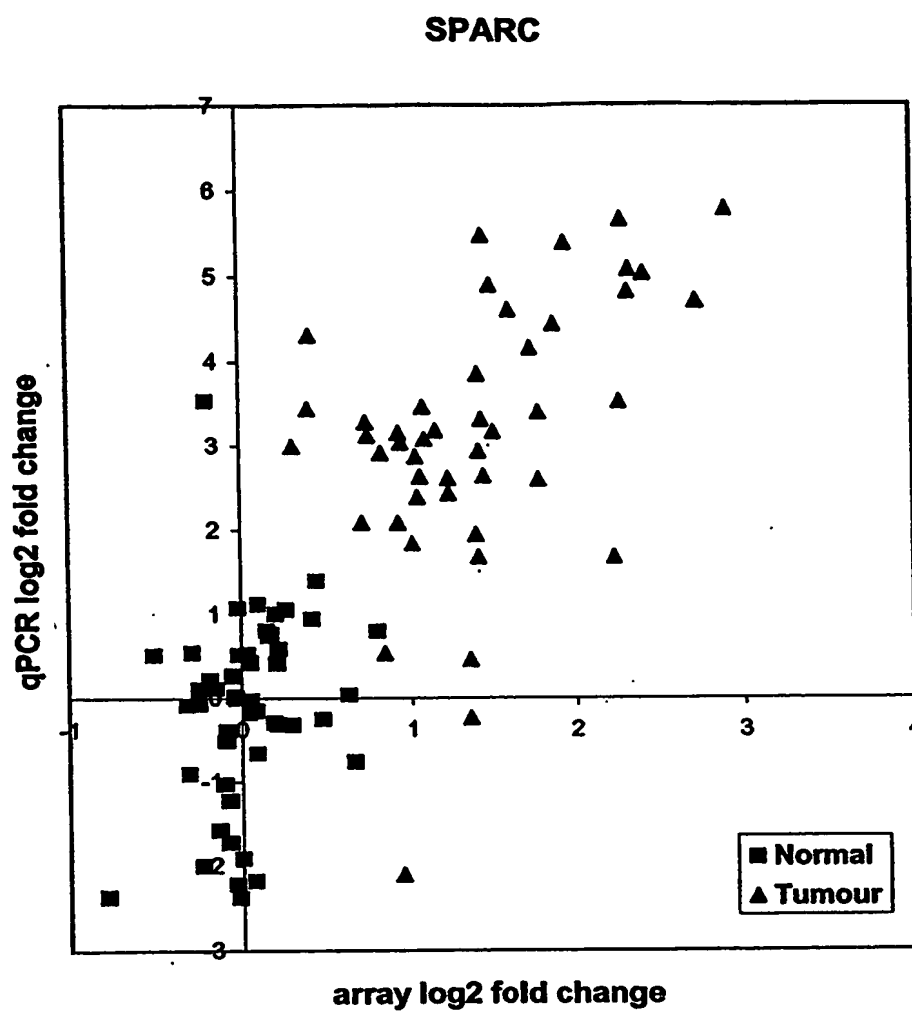
Figure 3

**Figure 4(a)**

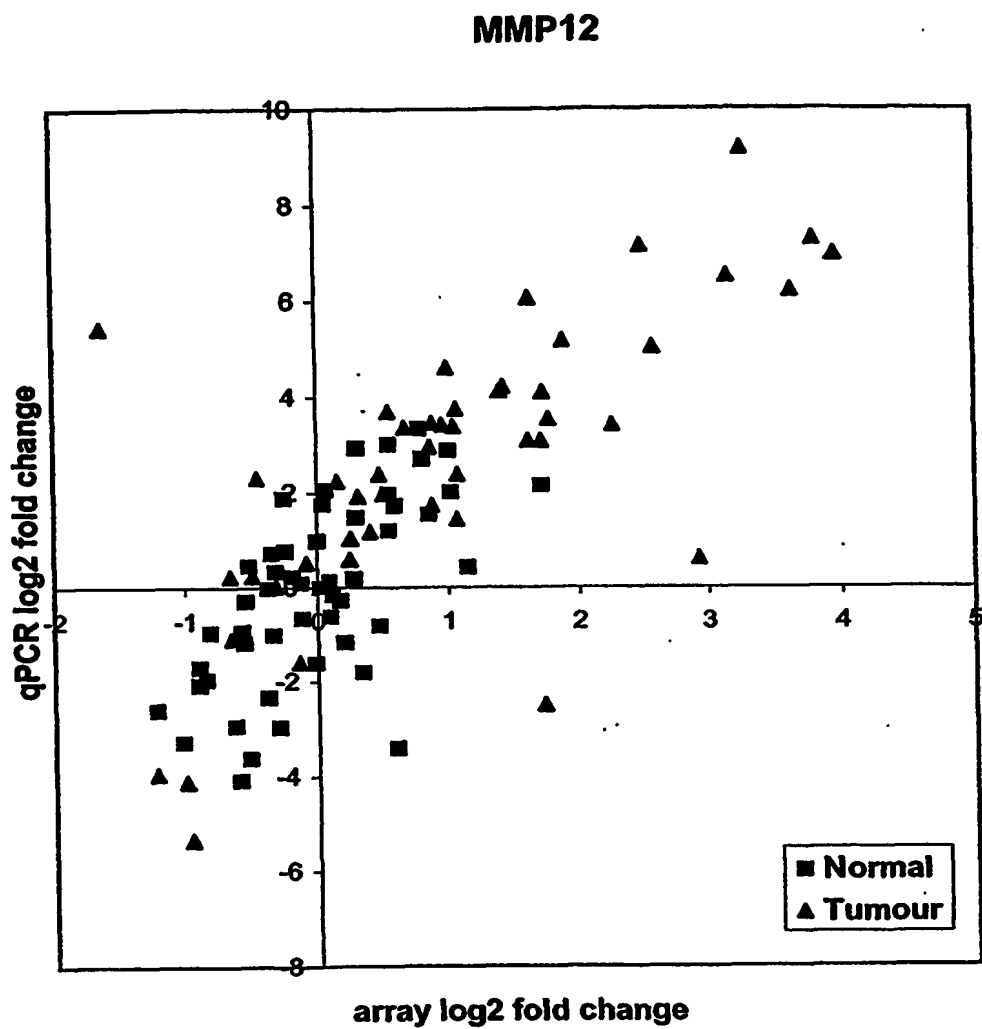
5/104

**Figure 4(b)**

6/104

**Figure 4(c)**

7/104

**Figure 4(d)**

ASPEN-tumor:median normal log2 fold change

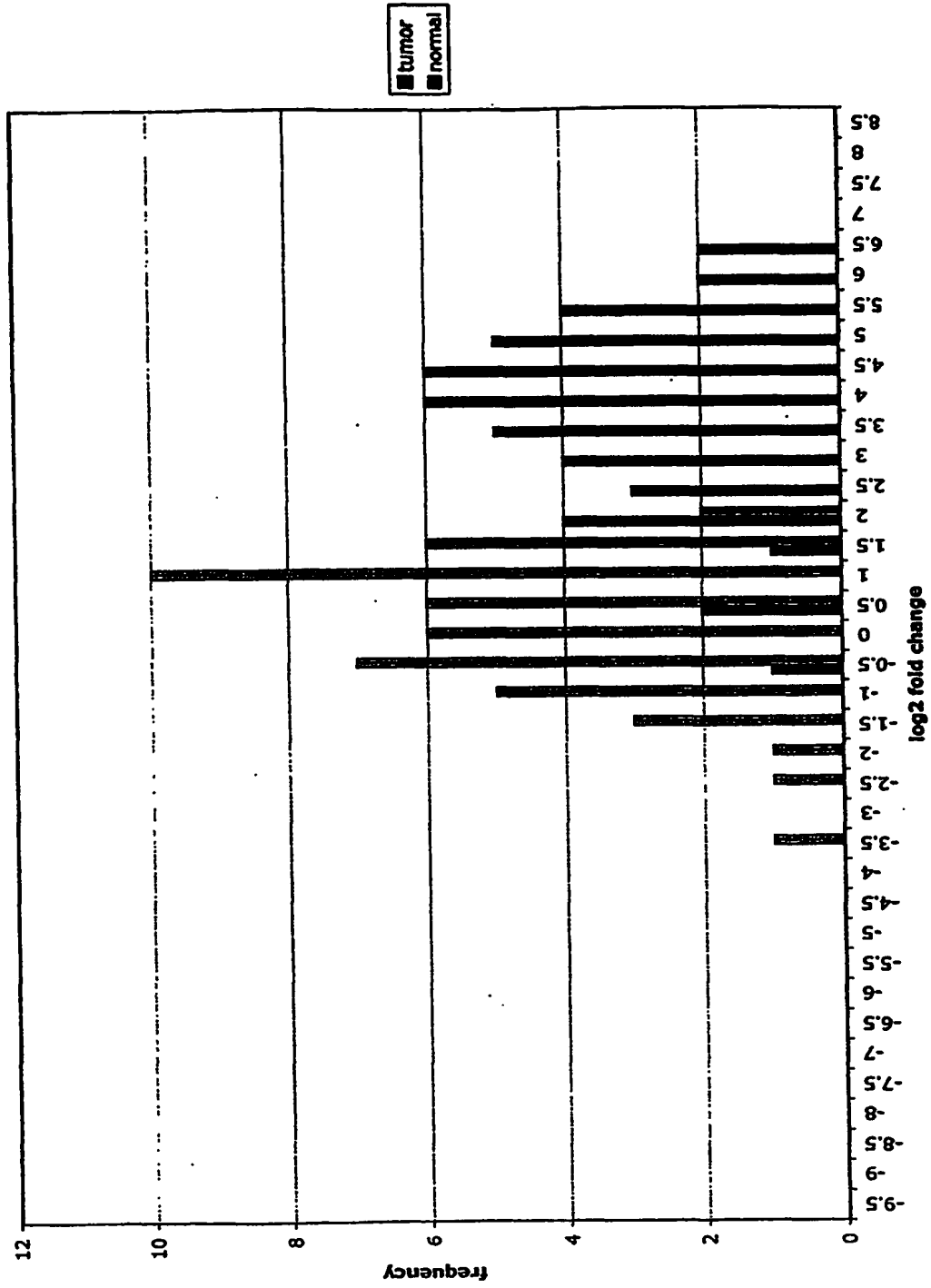


Figure 5(a)

CST1,2 & 4-tumor:median normal log2 fold change

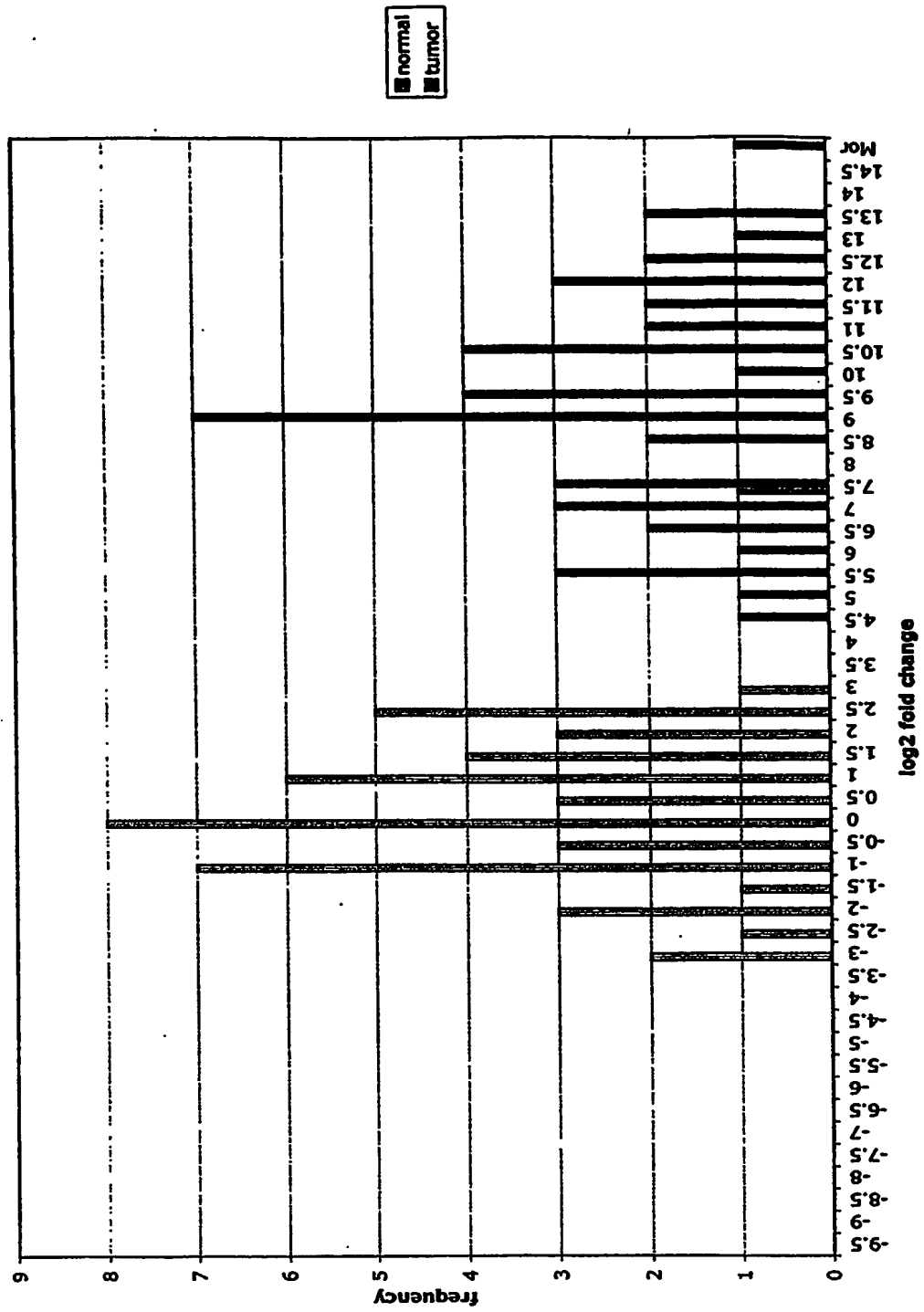


Figure 5(b)

10/104

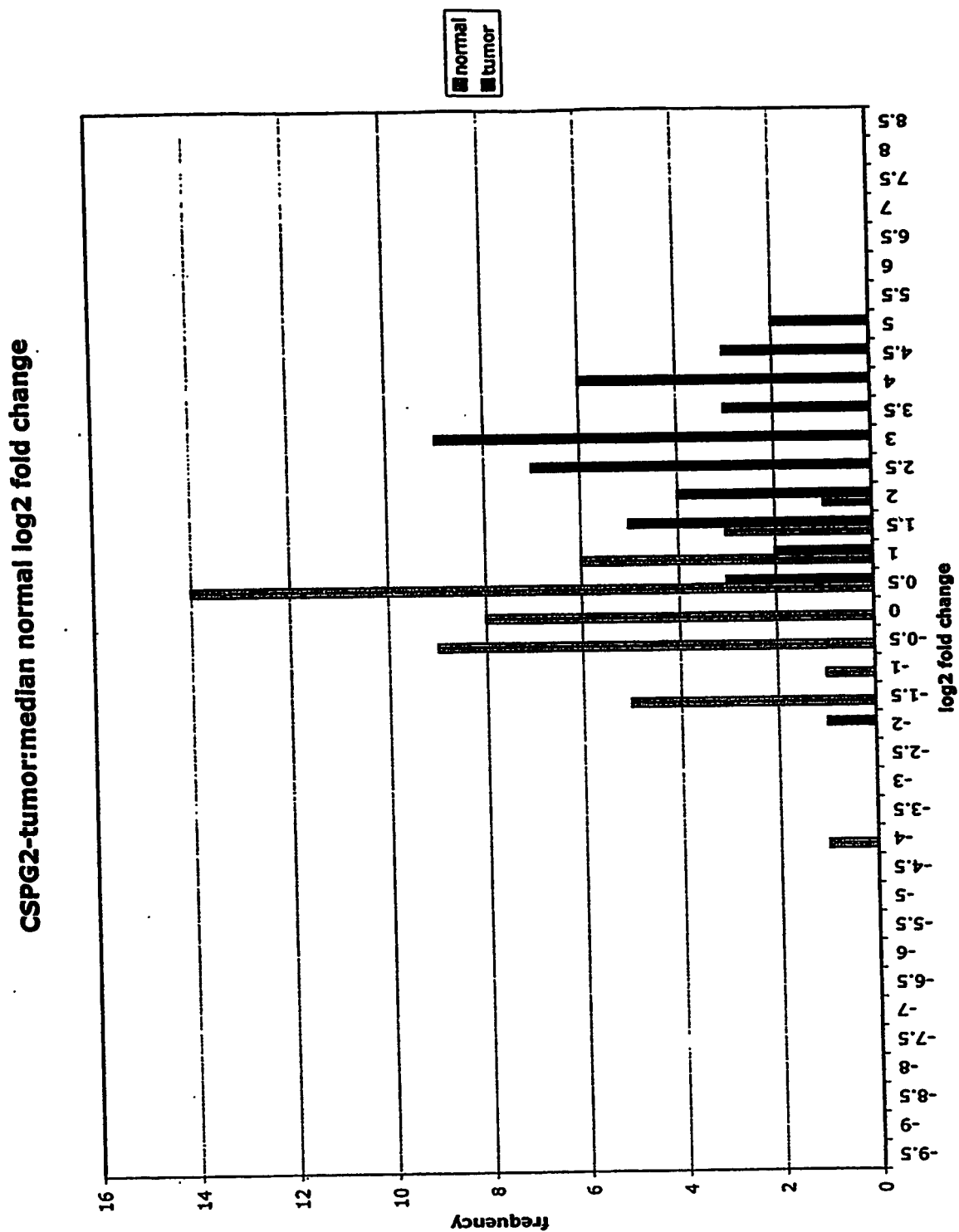


Figure 5(c)

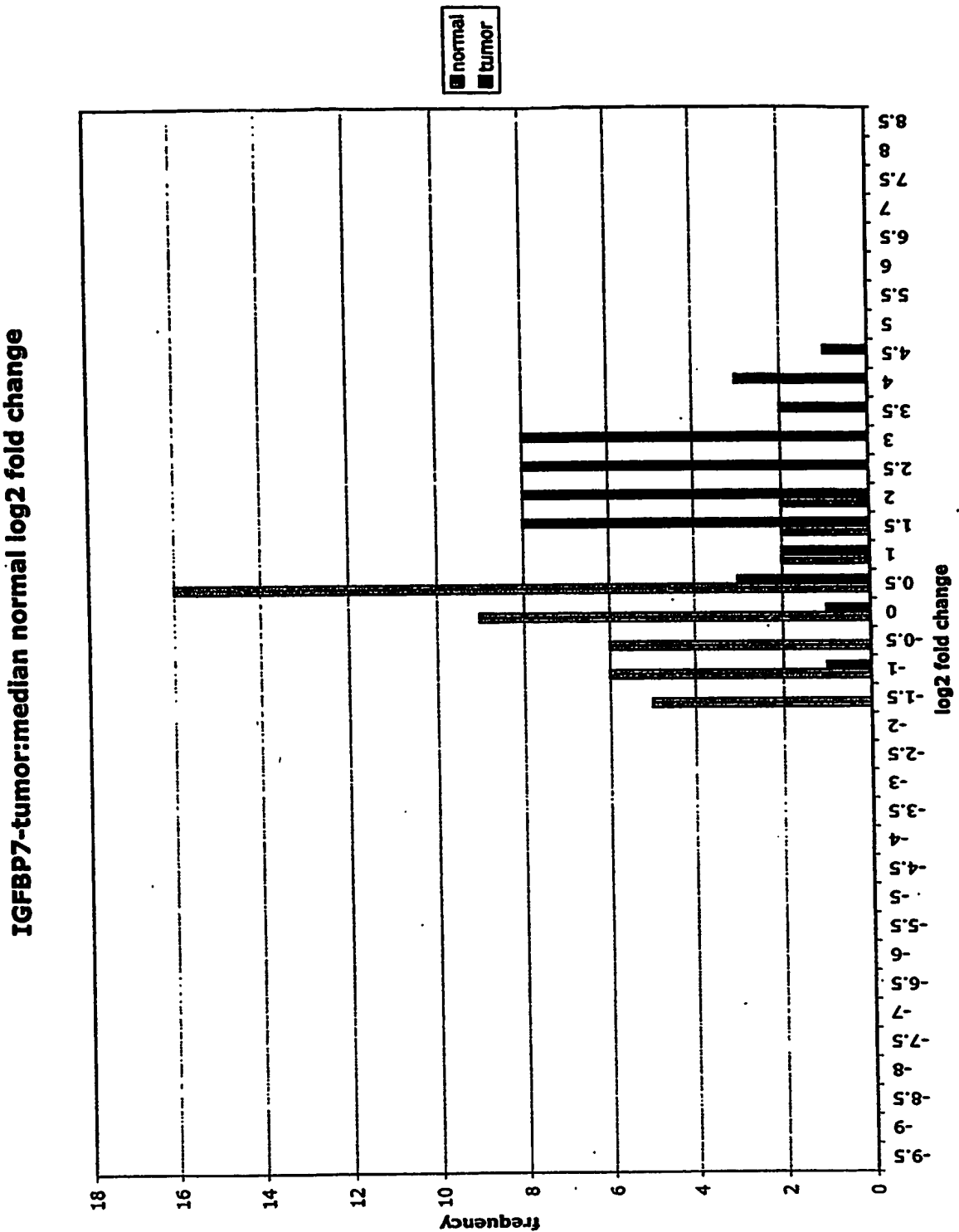


Figure 5(d)

12/104

INHBA-tumor:median normal log2 fold change

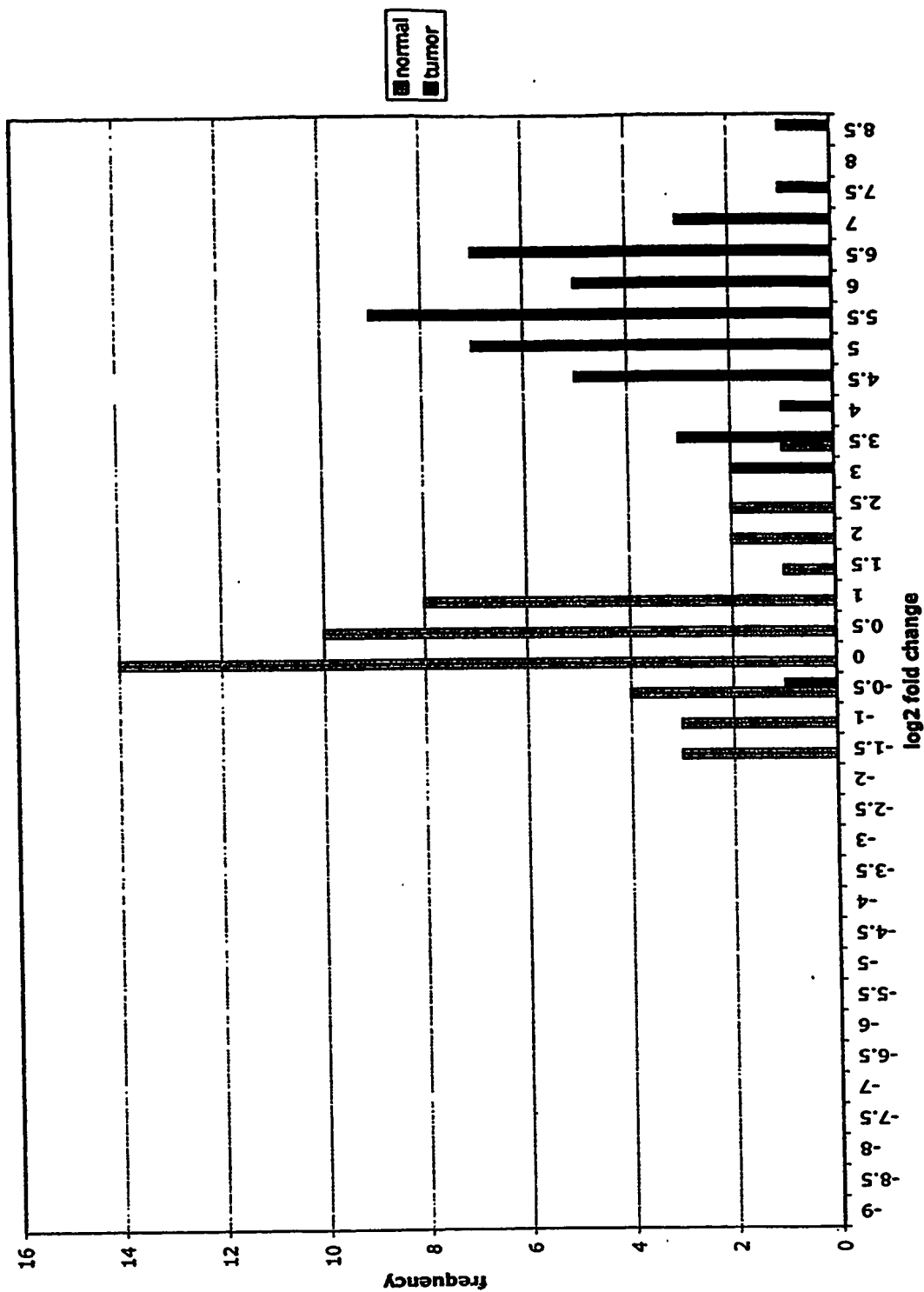


Figure 5(e)

LOXL2-tumor:median normal log2 fold changes

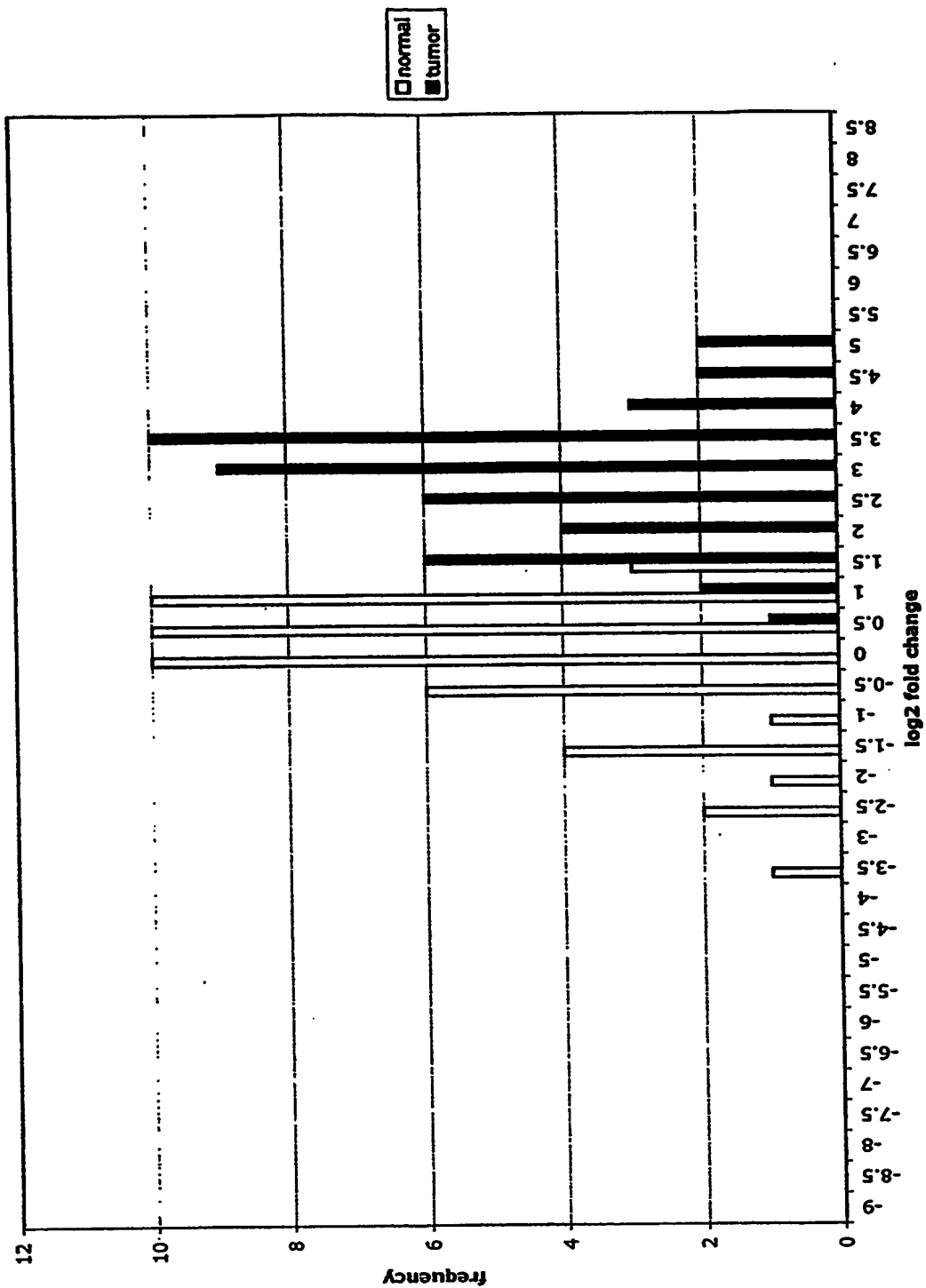


Figure 5(f)

lumican-Tumor:median normal log2 fold changes

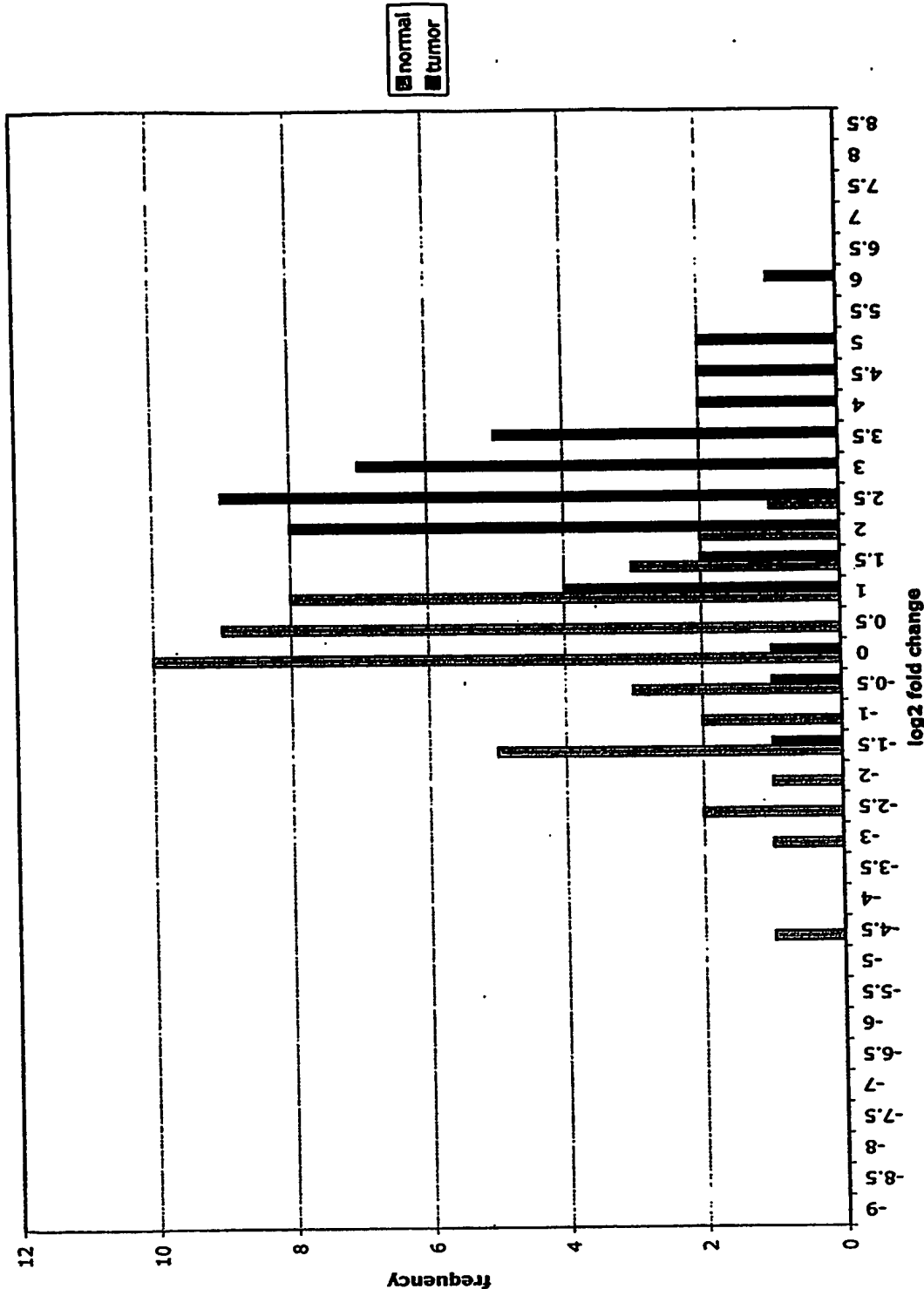


Figure 5(g)

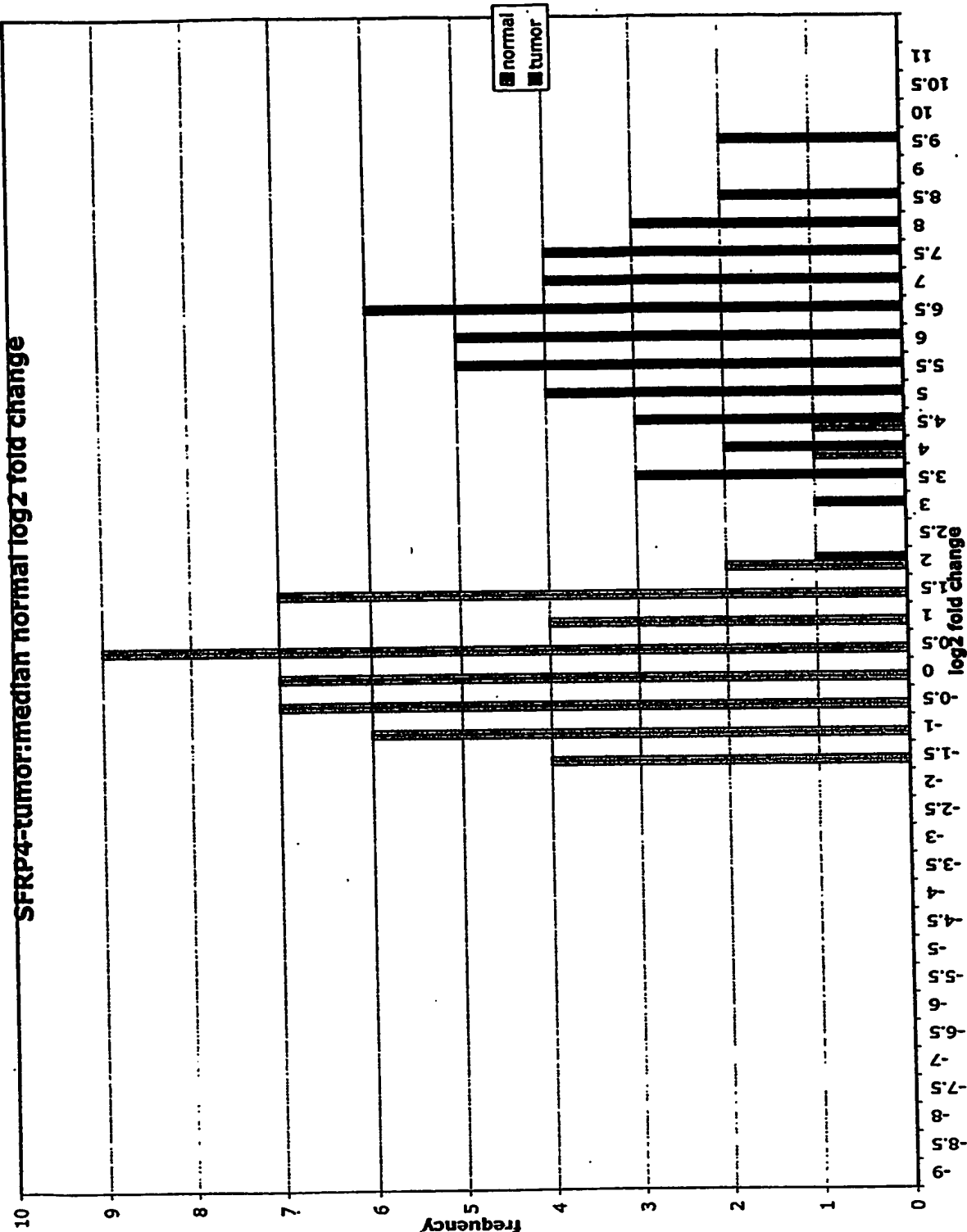


Figure 5(h)

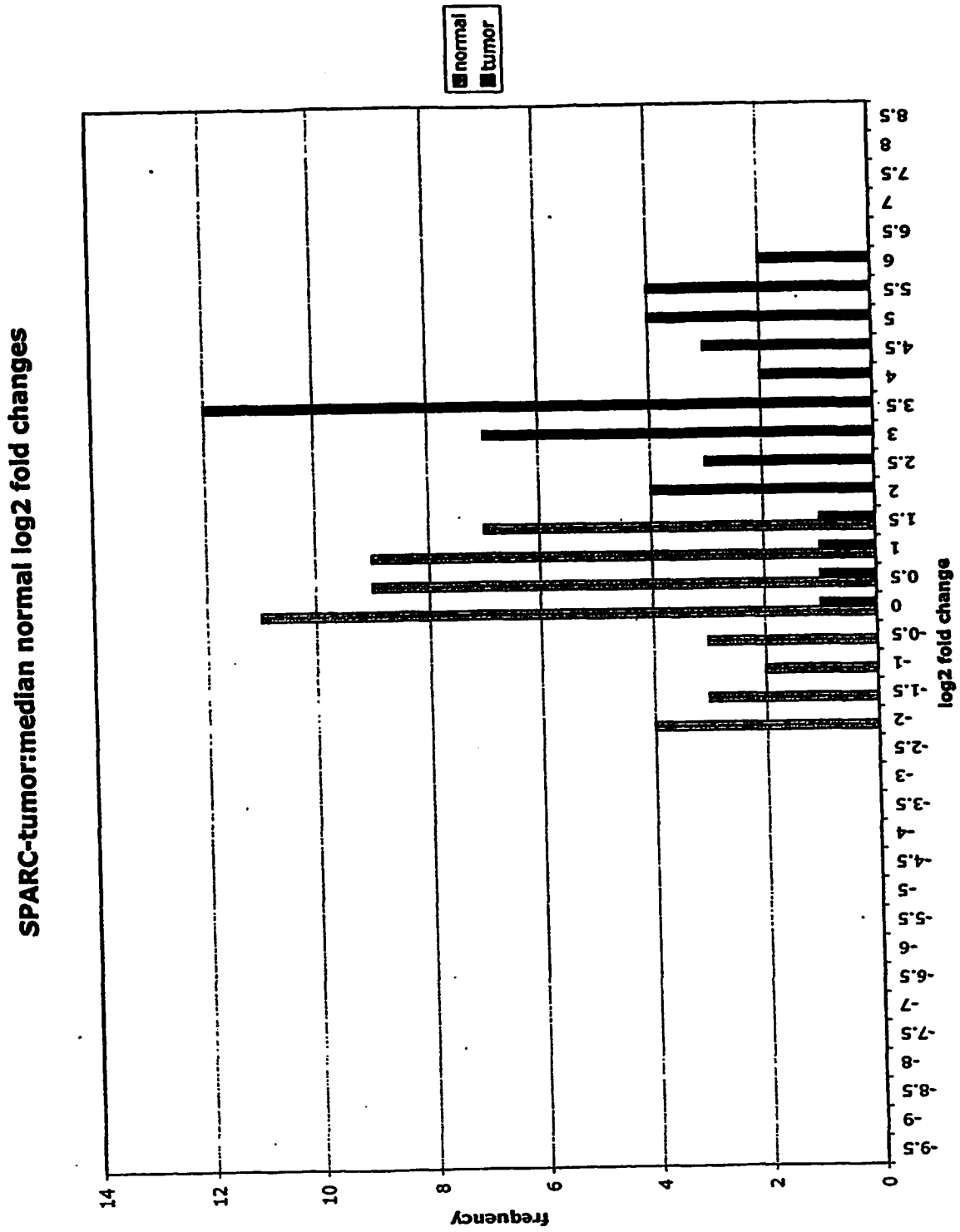


Figure 5(i)

17/104

SPP1-tumor:median normal log2 fold change

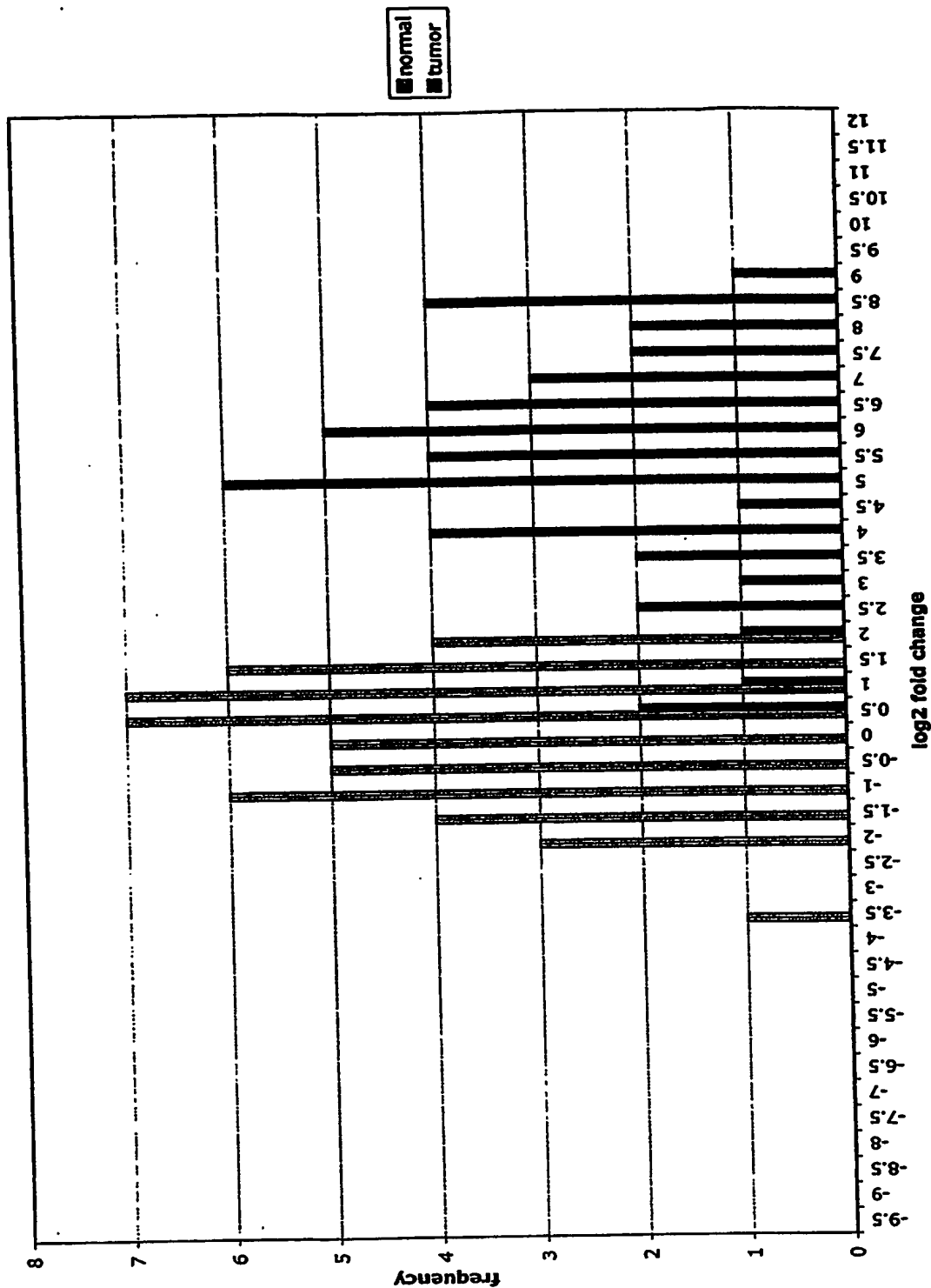


Figure 5(i)

18/104

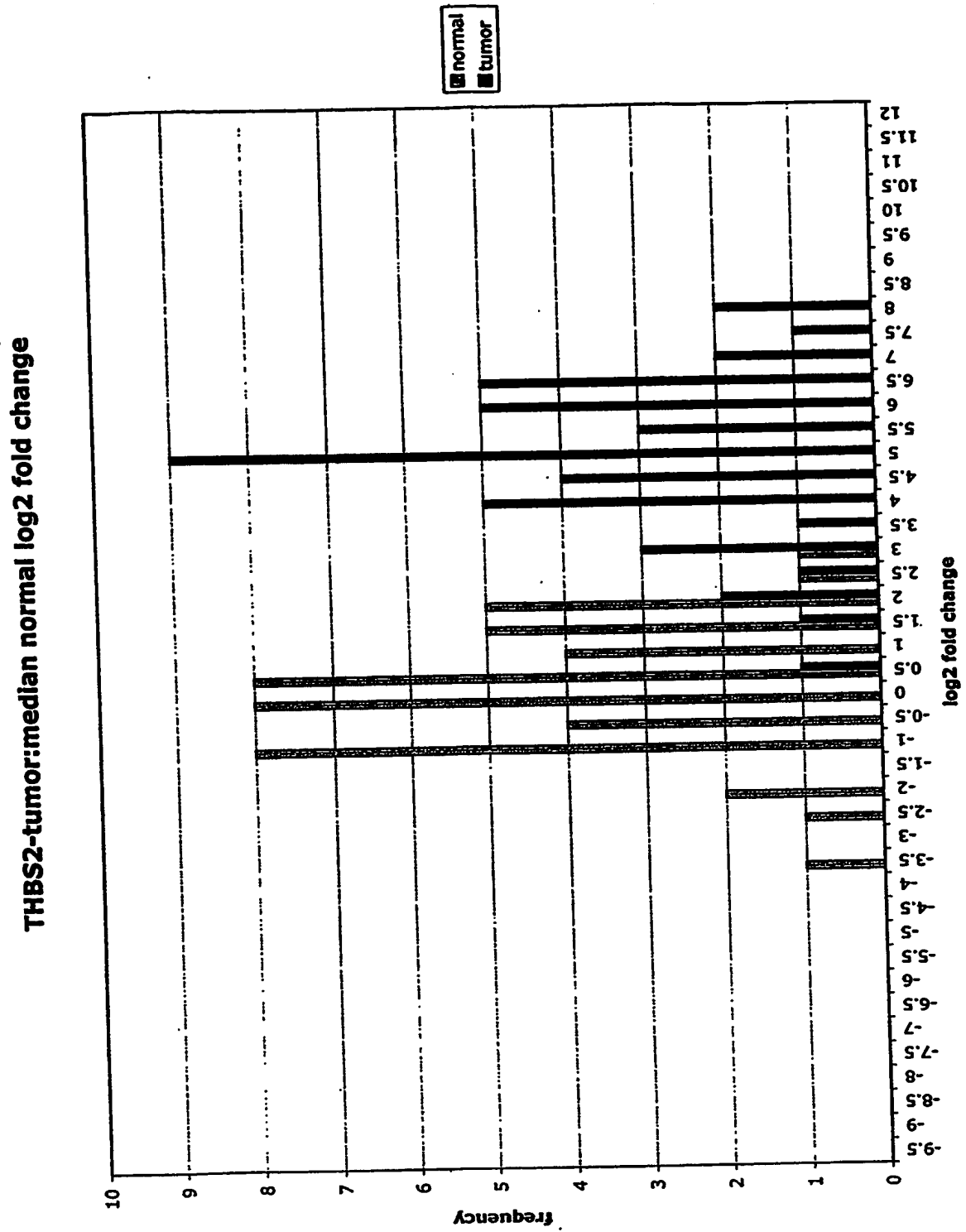


Figure 5(k)

TIMP1-tumor:median normal log2 fold change

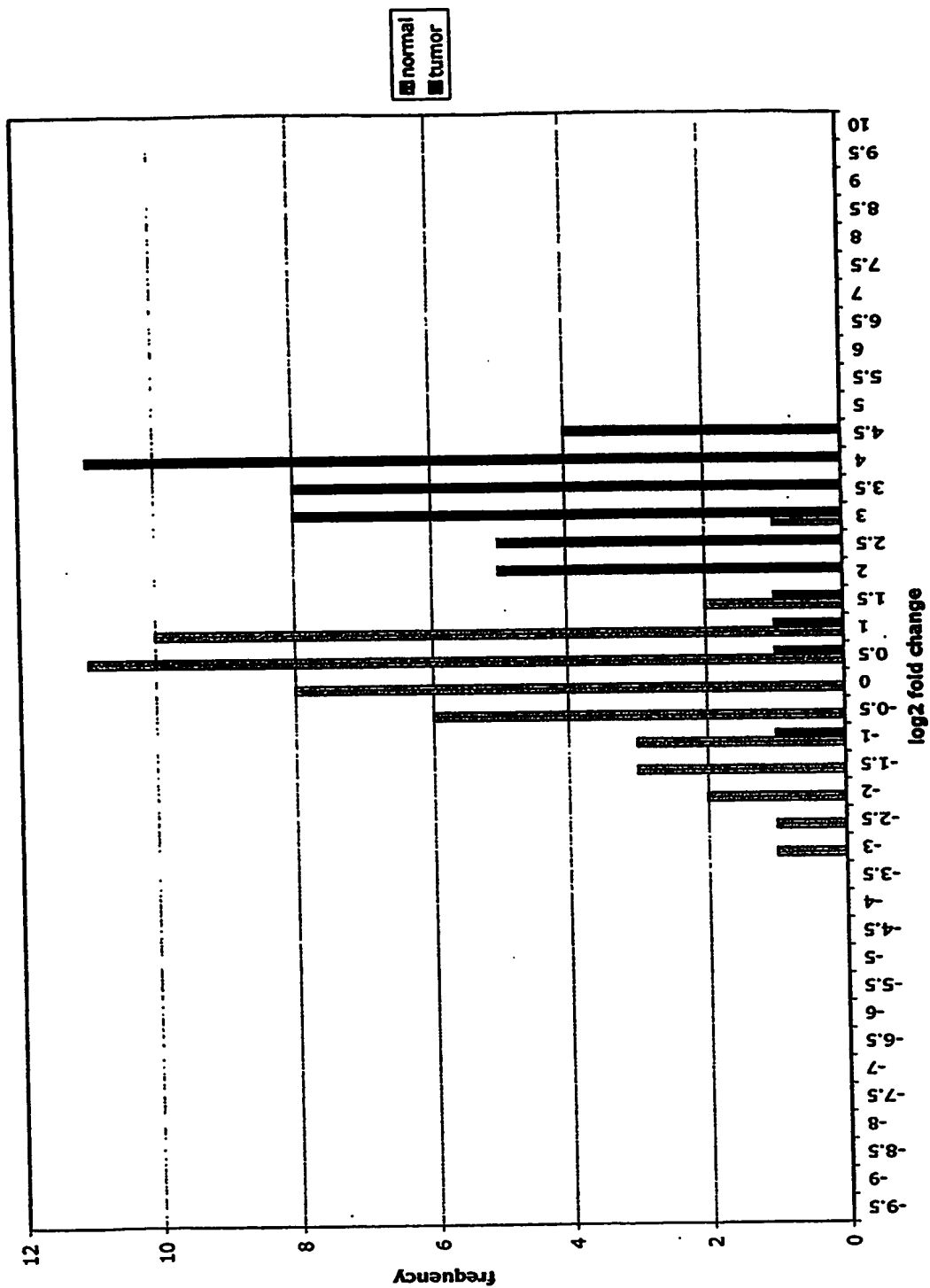


Figure 5(1)

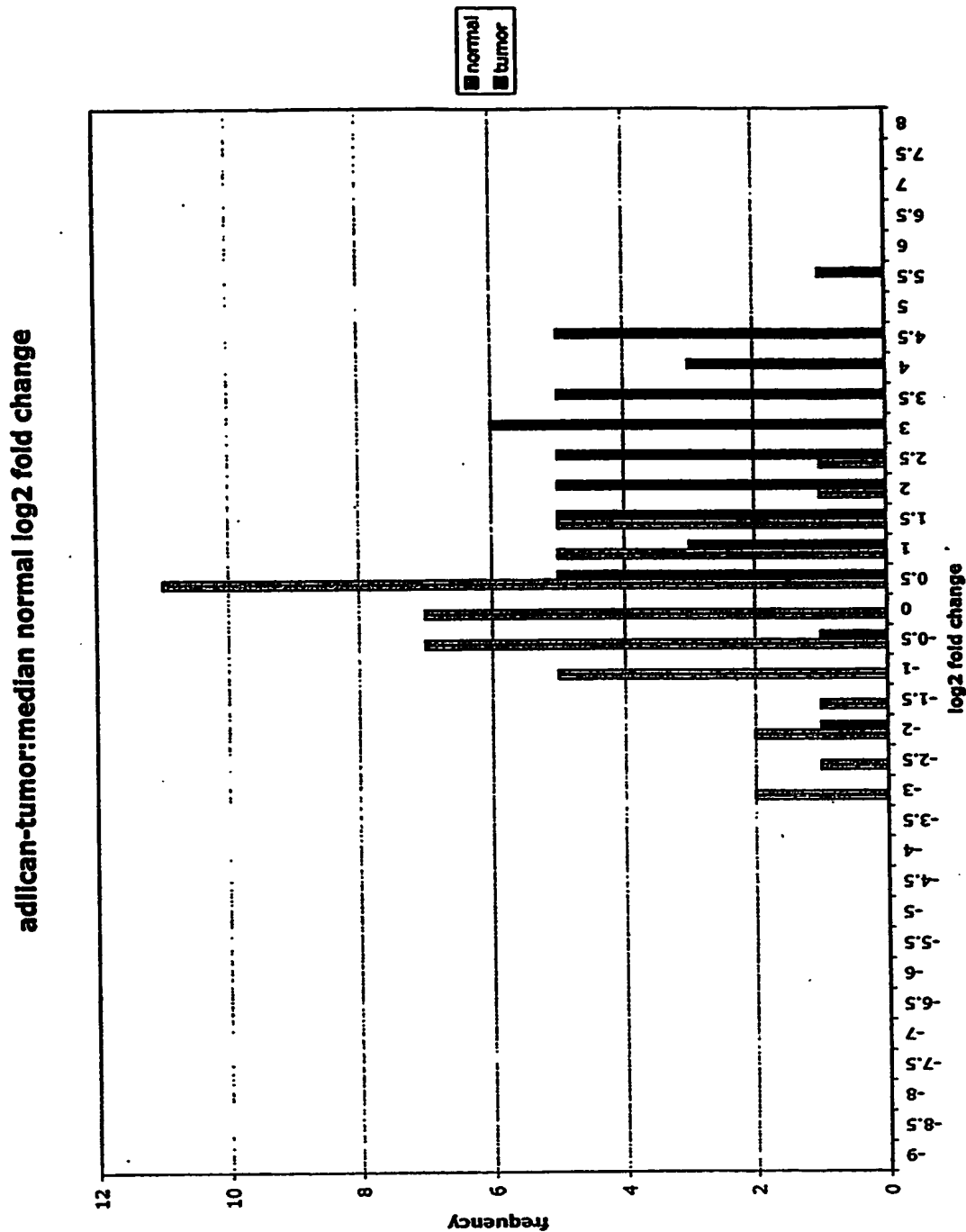


Figure 5(m)

PRS11- tumor:median normal log2 fold change

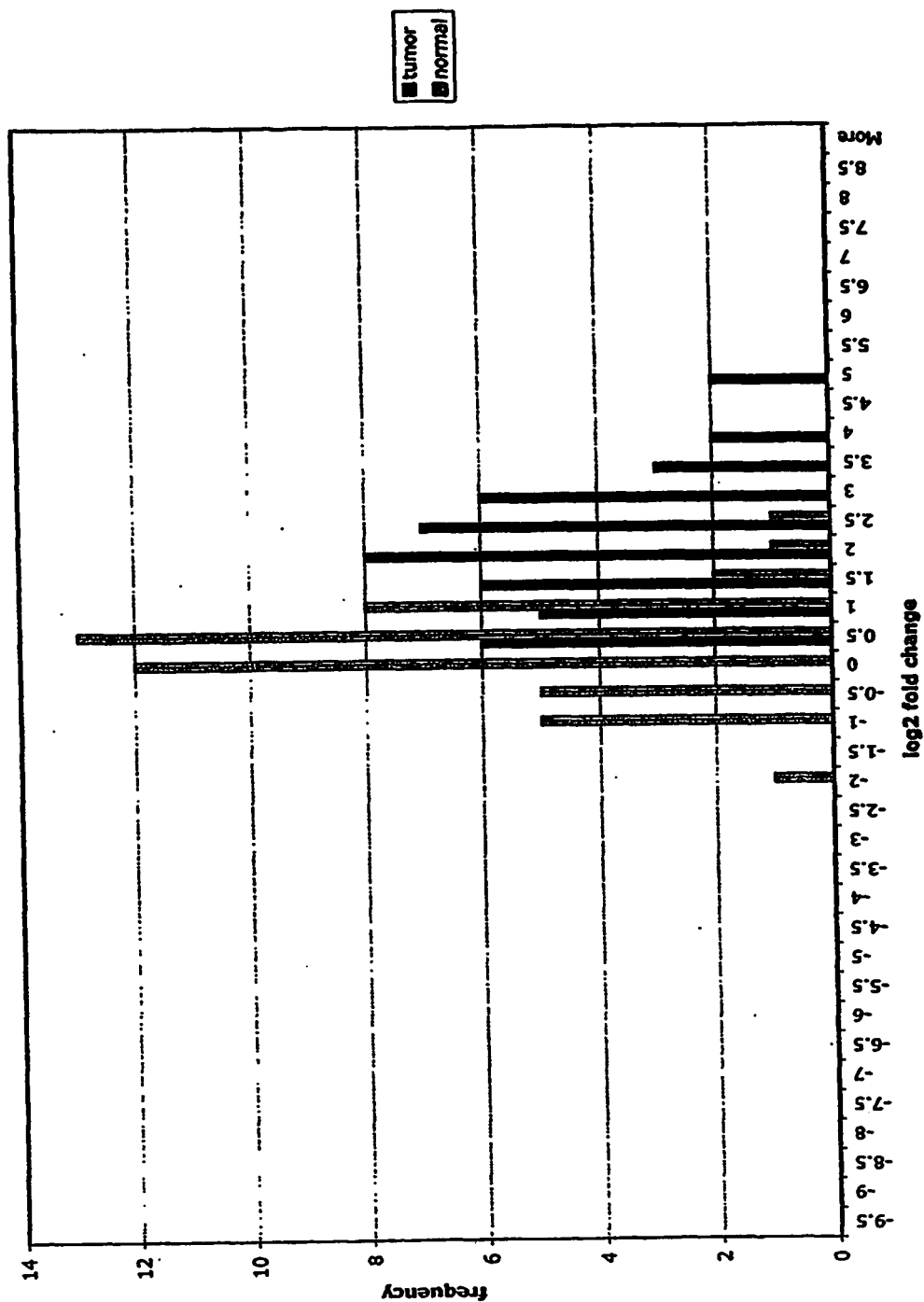


Figure 5(n)

ASAH1-tumor:median normal log2 fold changes

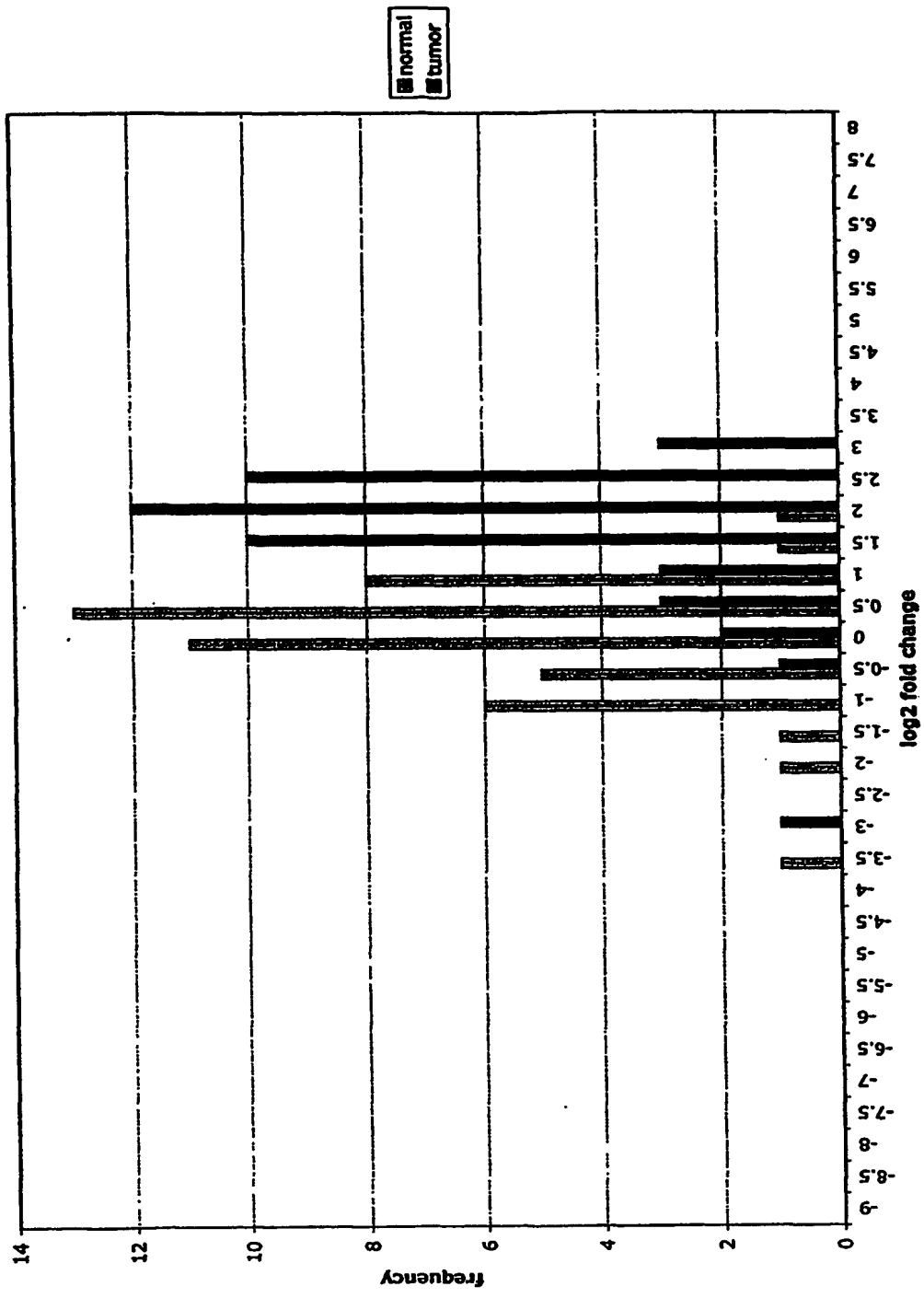


Figure 5(o)

SFRP2-tumor:median normal log2 fold change

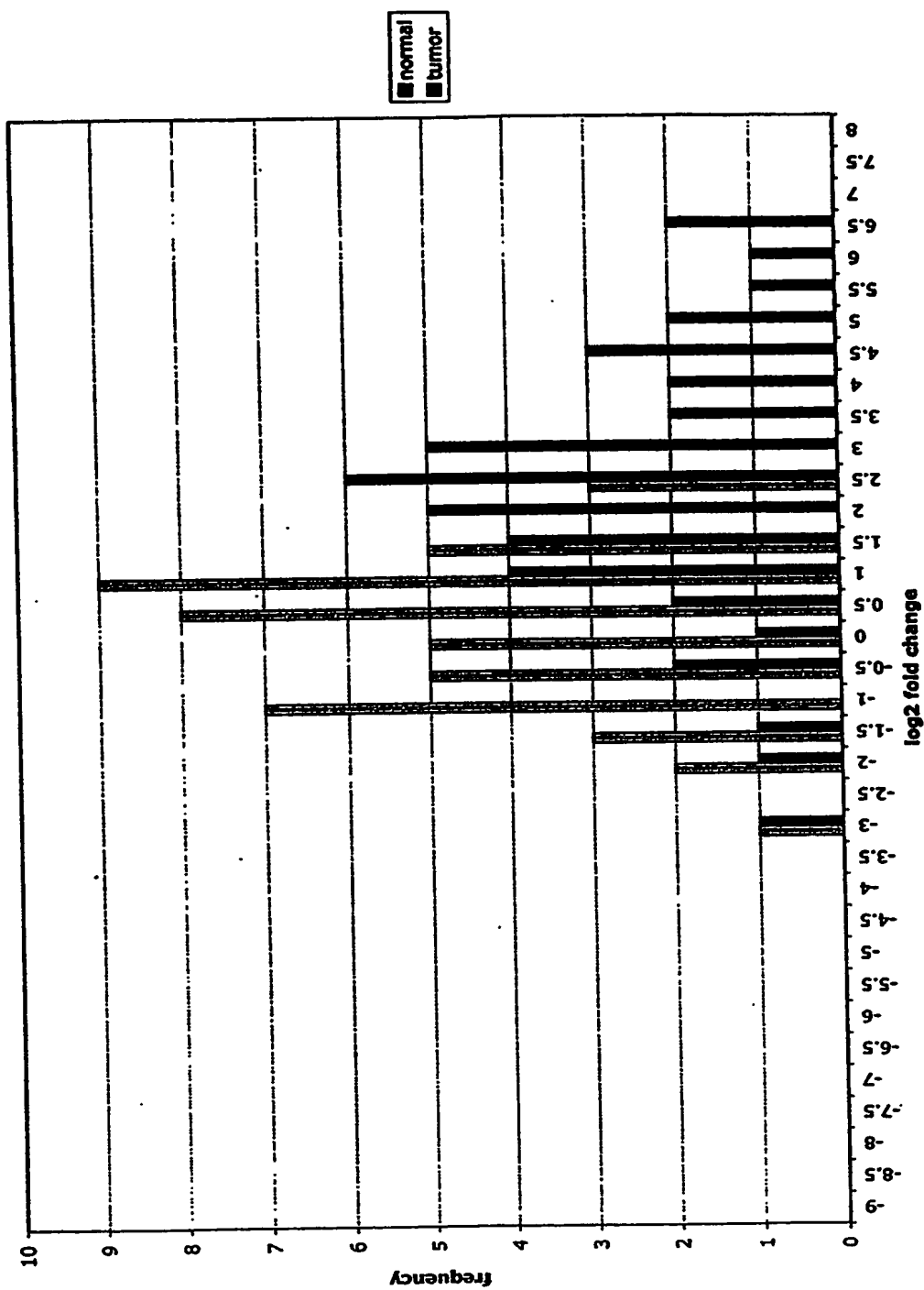


Figure 5(p)

24/104

GGH-tumor:normal log2 fold change

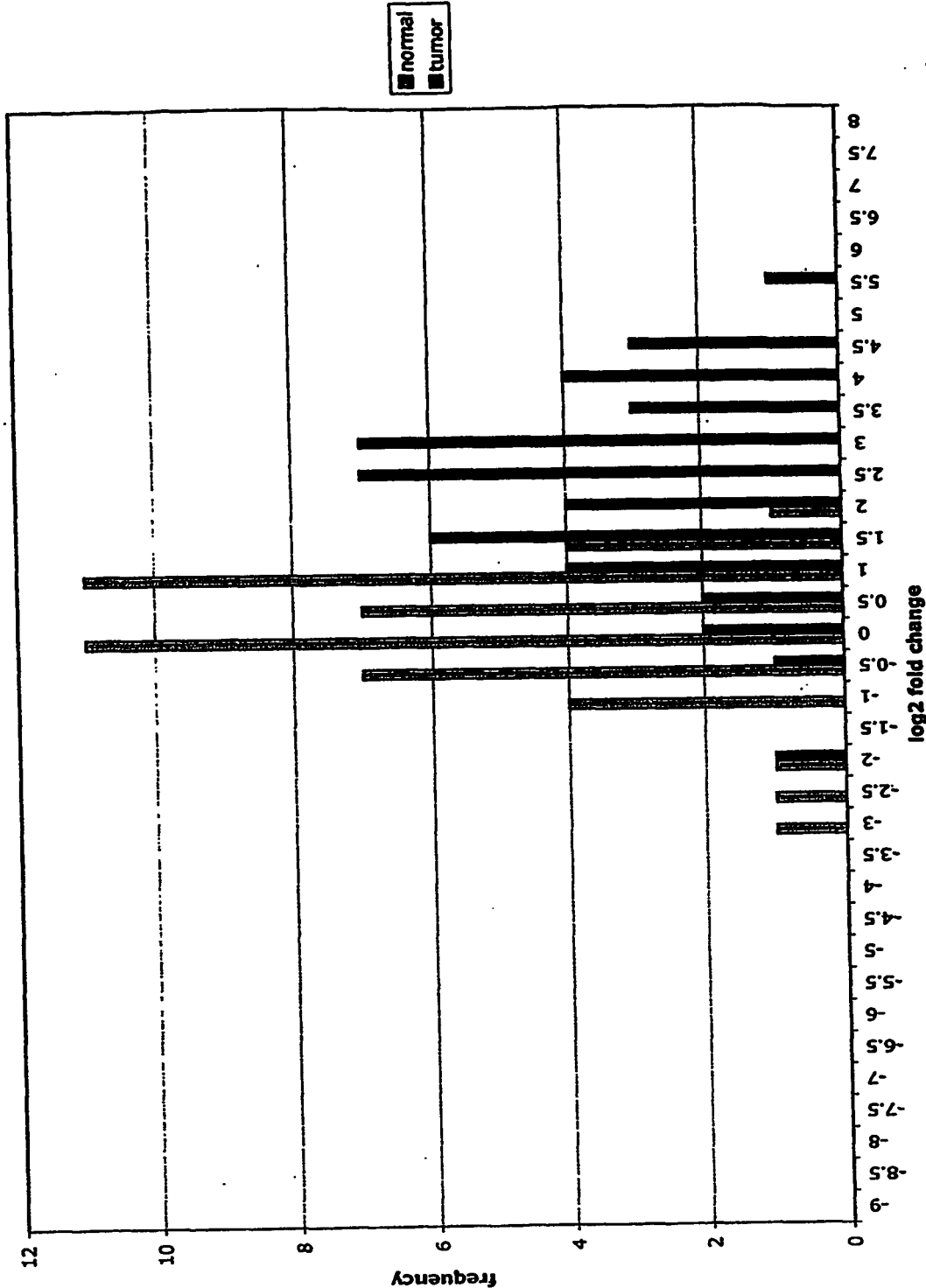


Figure 5(q)

MMP12-tumor:median normal log2 fold changes

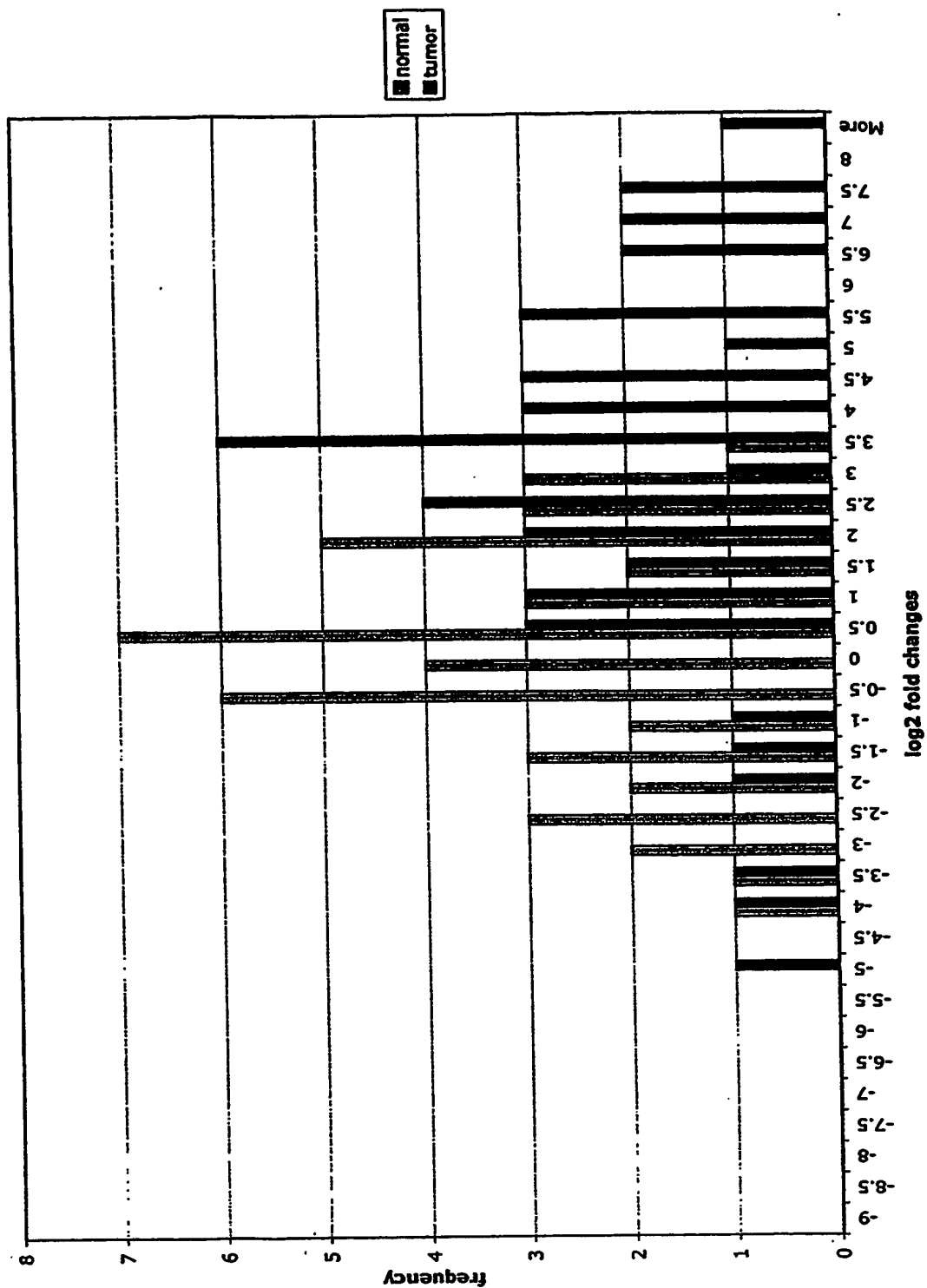


Figure 5(r)

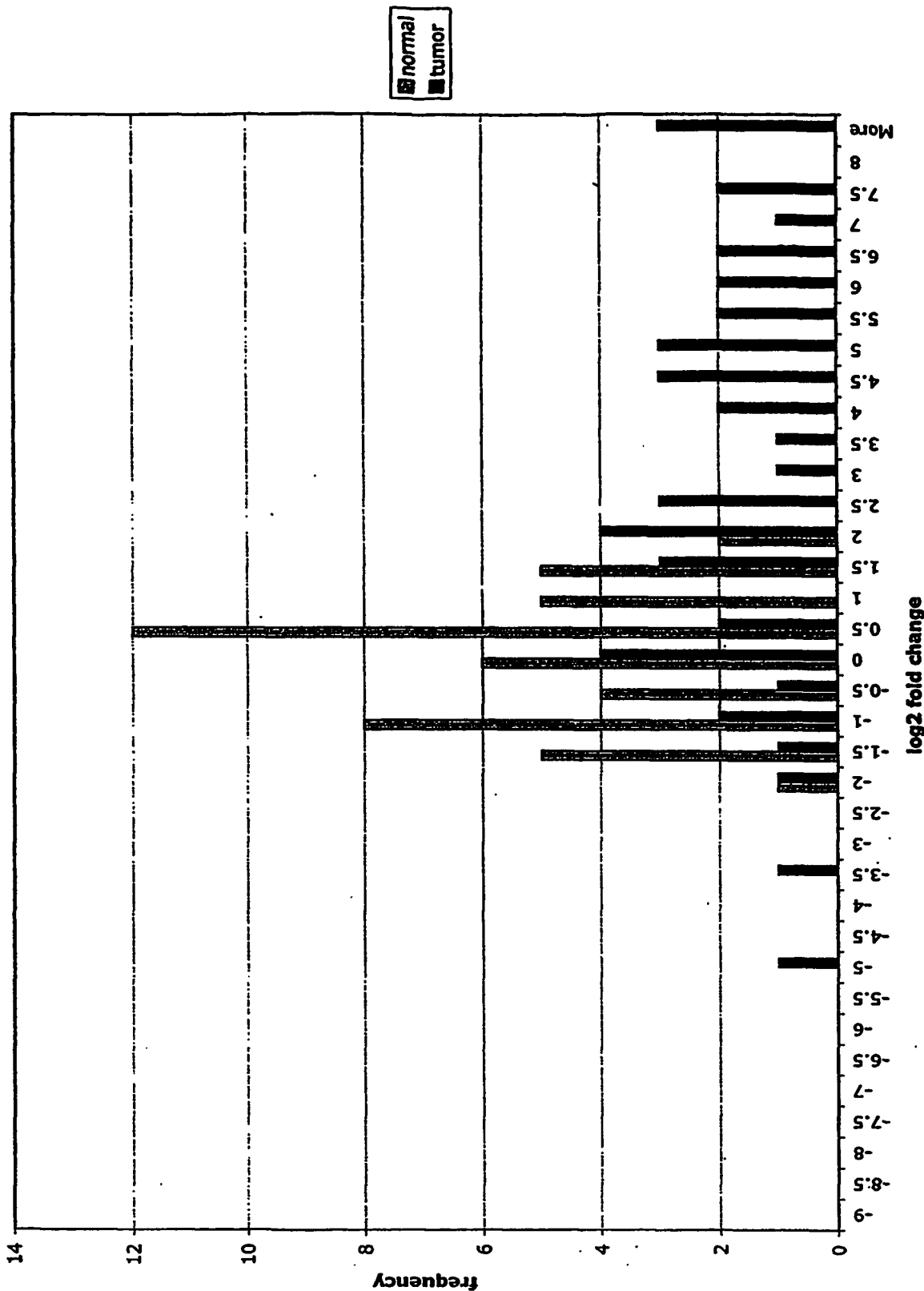


Figure 5(s)

LEPRE1-tumor:median normal log2 fold changes

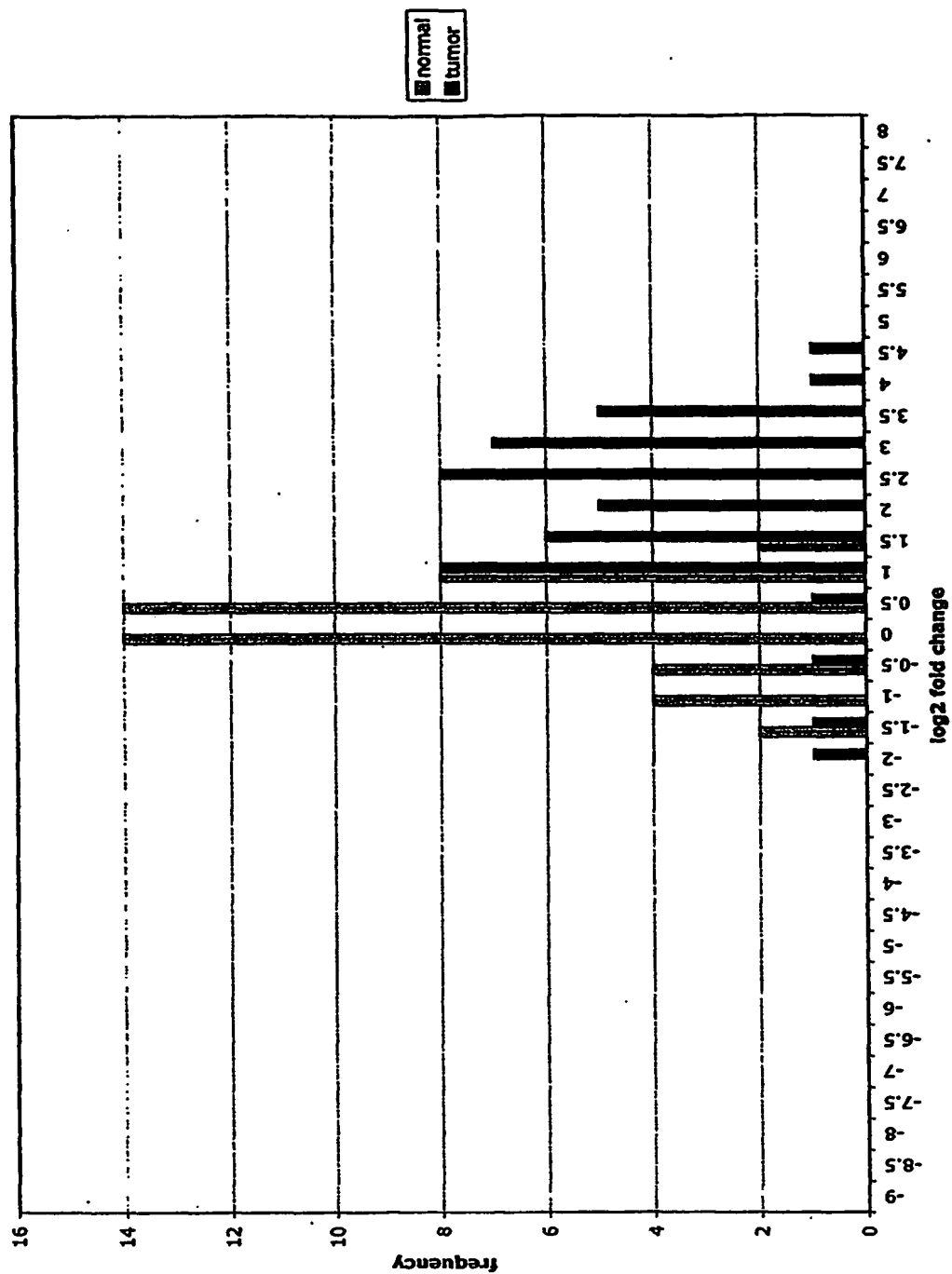


Figure 5(t)

TG-tumor:median normal log2 fold change

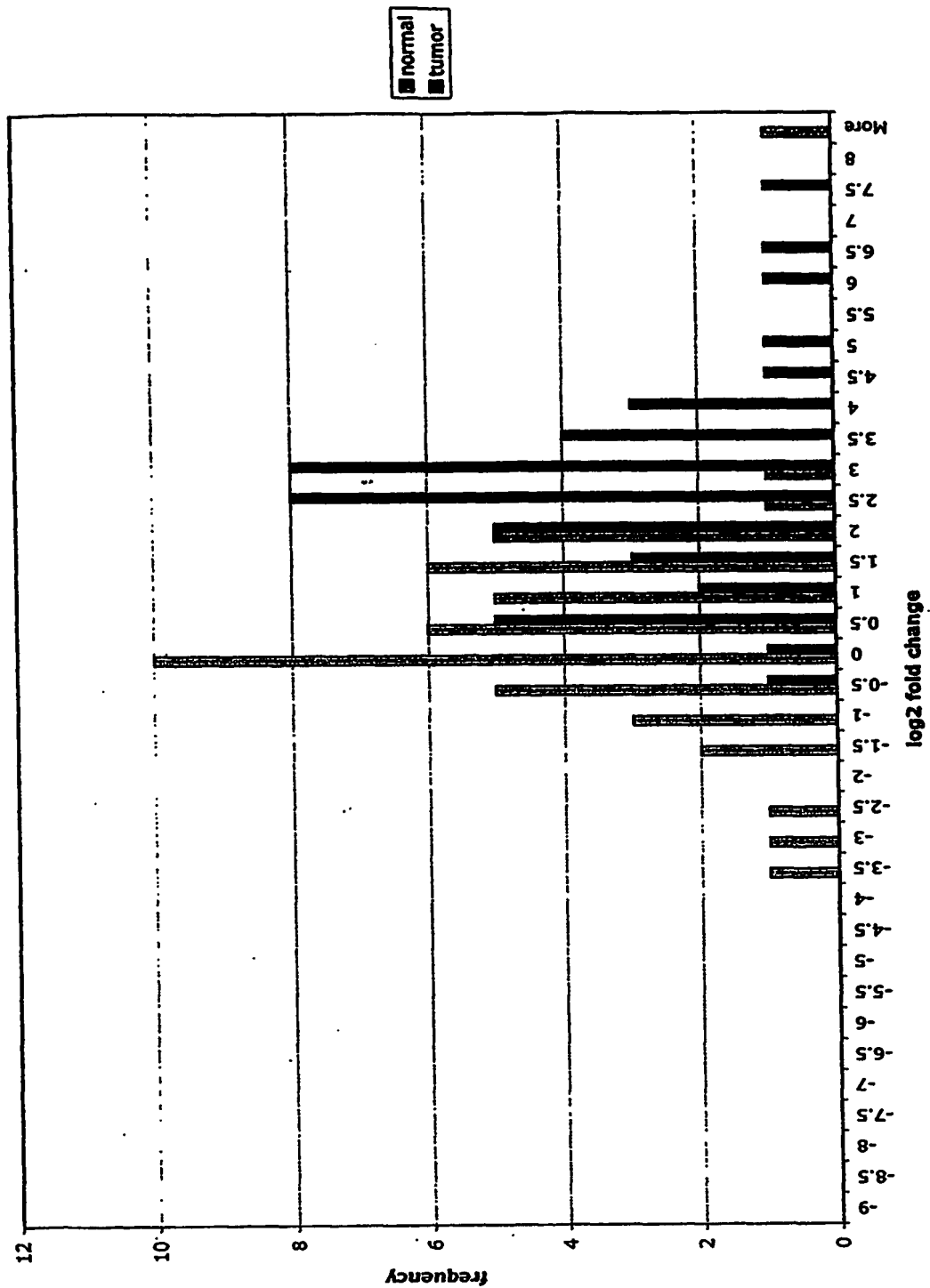


Figure 5(u)

29/104

EFEMP2-tumor:median normal log2 fold change

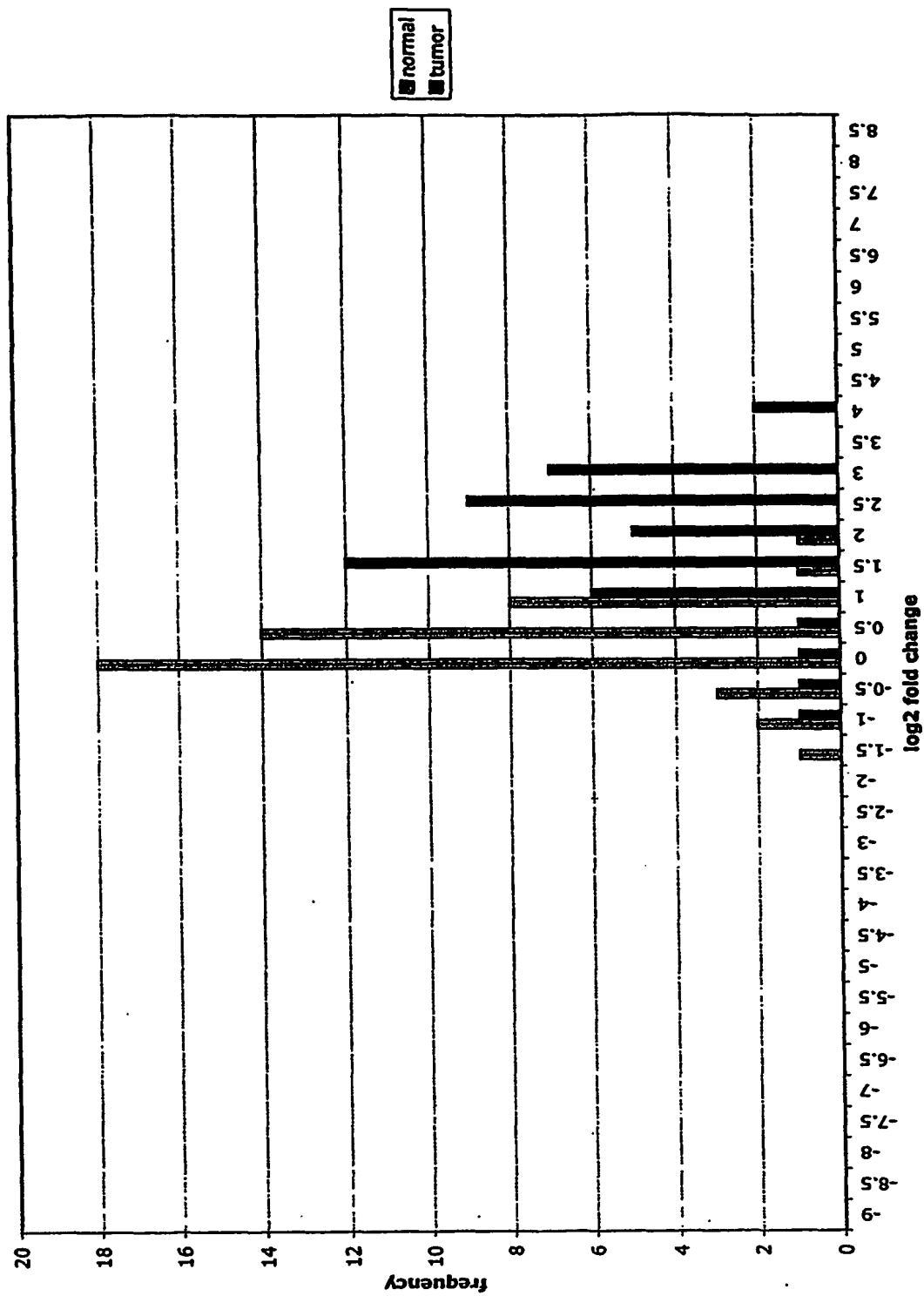


Figure 5(v)

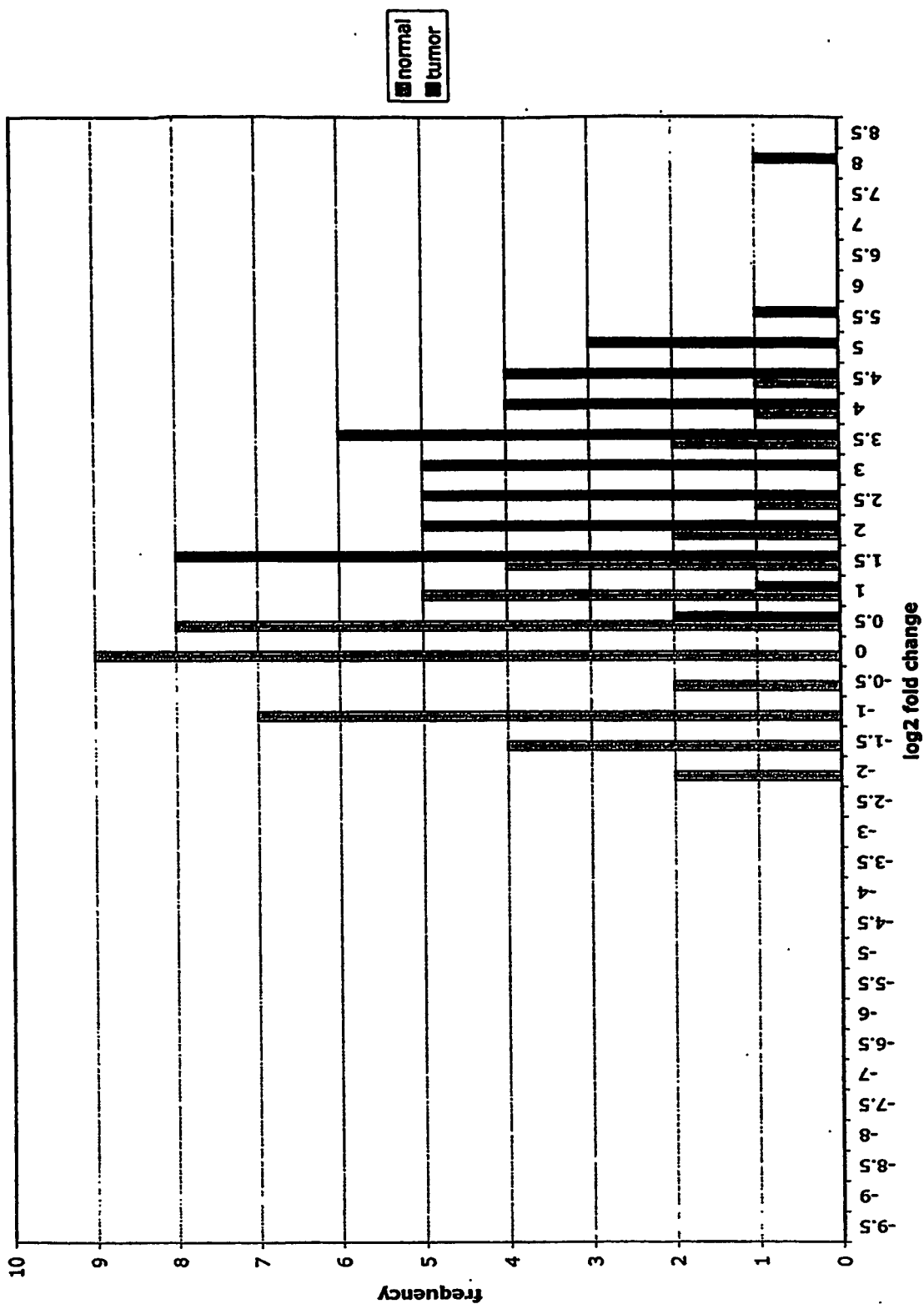


Figure 5(w)

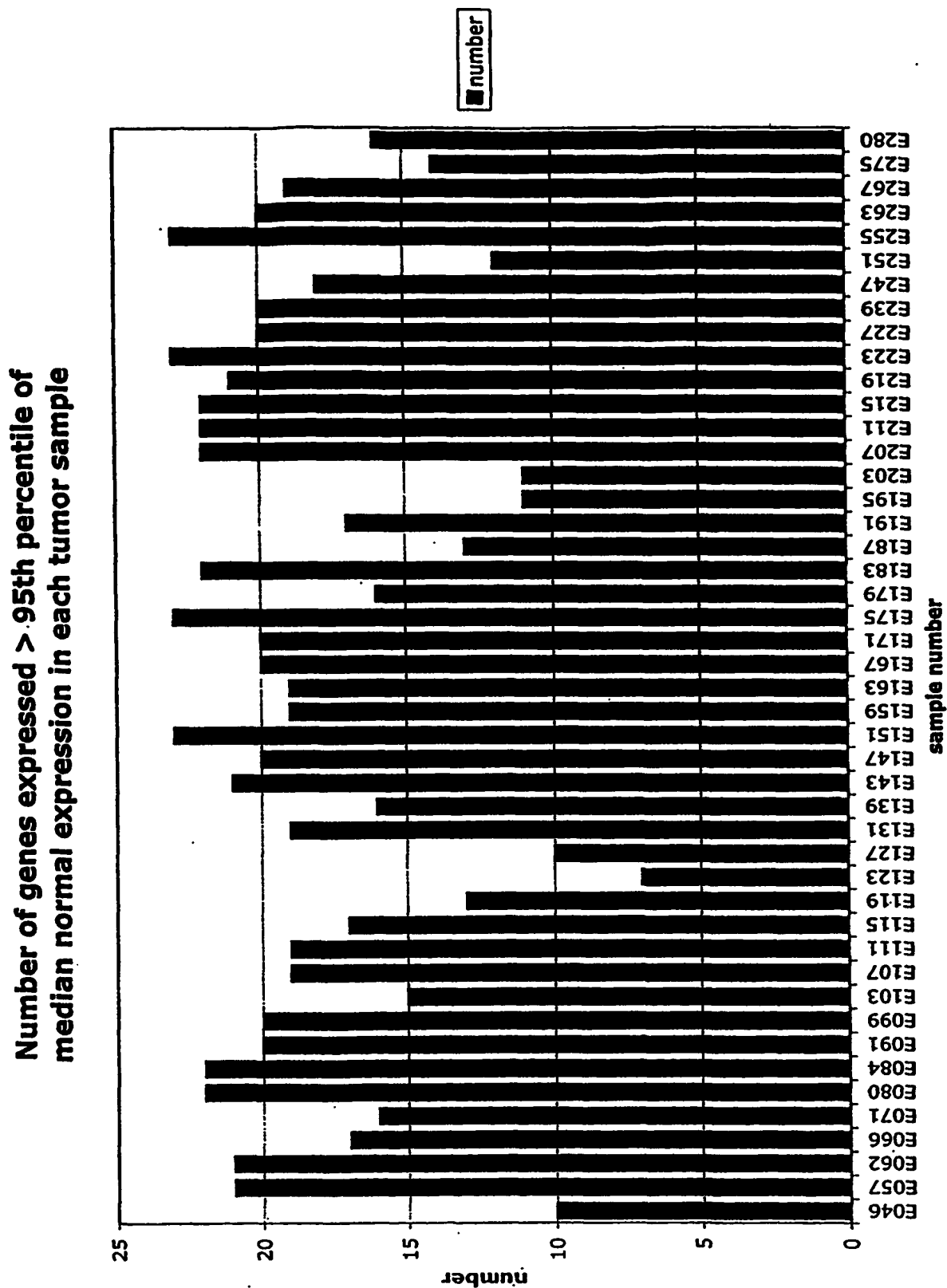


Figure 6

BEST AVAILABLE COPY

Fig.7a Relative expression of markers in tumor and normal samples compared to CEA

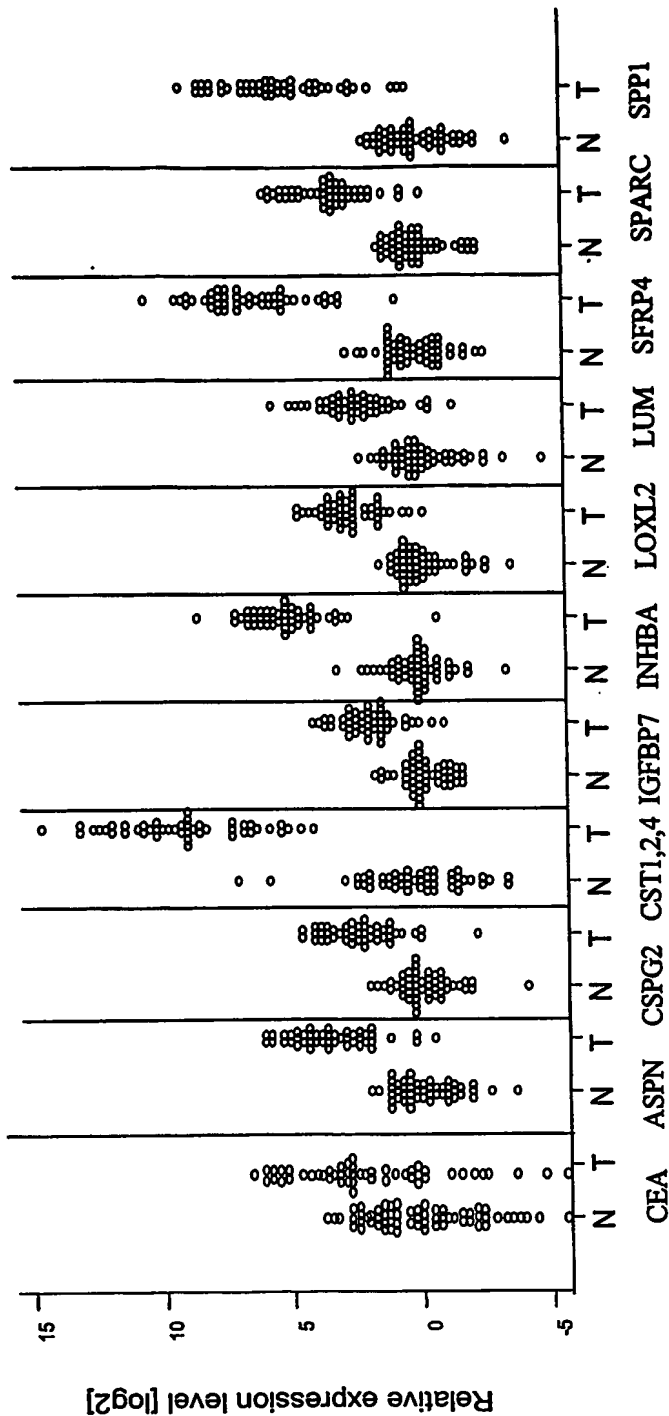


Fig. 7b

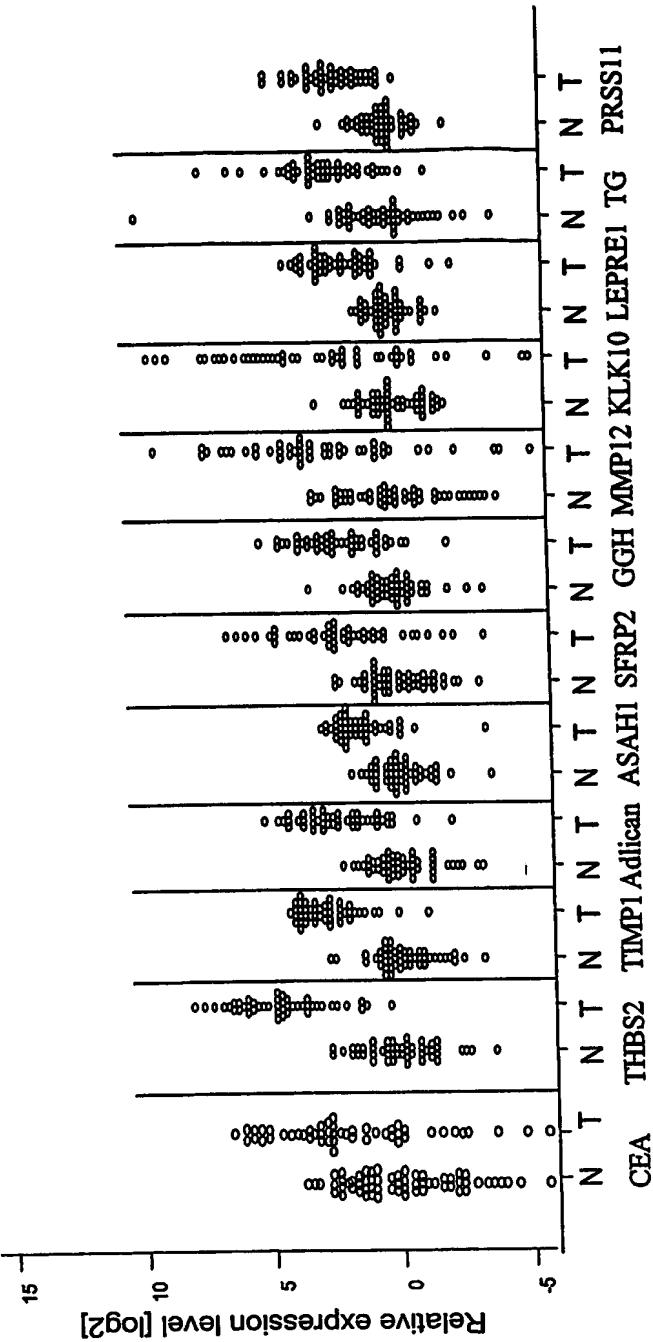


Fig. 7c

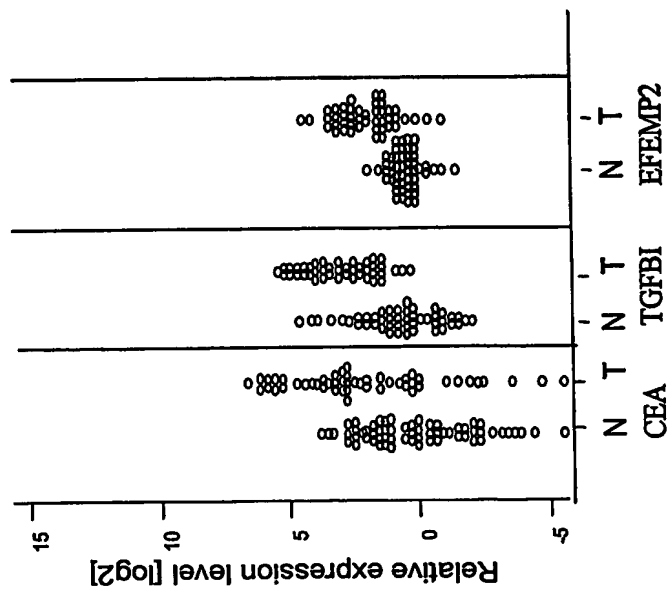


Fig. 8. Quantitative RT-PCR: expression in paired tumor and non-malignant samples of selected gastric cancer markers

name	symbol	median T:N fold change	maximum T:N fold change	% tumor samples with expression > paired non-malignant sample
adipon				
asporin (lr class 1)	ASPN	5	146	88
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	11	198	100
Cystatins SN, SA & S	CST1, 2, 4	5	68	93
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	498	11911	100
gamma-glutamyl hydrolase	GGH	3	17	93
Inhibin beta A chain	INHBA	4	34	83
Insulin-like growth factor binding protein 7	IGFBP7	27	630	95
Kallikrein 10	KLK10	5	38	93
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1	7	519	78
lumican	LUM	4	23	85
lysyl oxidase-like 2	LOXL2	5	68	90
matrix metalloproteinase 12	MMP12	7	53	95
metalloproteinase inhibitor 1	TIMP1	9	468	85
n-acylsphingosine amidohydrolase	ASAH1	6	103	95
osteopontin	SPP1	3	15	88
secreted frizzled-related protein 2	SFRP2	36	626	98
secreted frizzled-related protein 4	SFRP4	5	48	83
secreted protein, acidic, cysteine rich	SPARC	54	375	100
serine protease 11 (IGF binding)	PRSS11	10	66	95
thrombospondin 2	THBS2	4	63	90
thyroglobulin	TG	23	452	98
transforming growth factor B-induced	TGFB1	4	174	93
cell growth regulatory factor with EF-hand domain	CGR11	5	78	95
serine (or cysteine) proteinase inhibitor H1	SERP1NH1	3	33	75
matrix metalloproteinase 12	MMP2	10	51	98
proprotein convertase subtilisin/kexin type 5	PCSK5	2	46	83
serine (or cysteine) proteinase inhibitor B5	SERP1NB5	2	63	80
transforming growth factor beta 1	TGFB1	5	861	73
		3	16	88
cardioembryonic antigen (CEA)	CEACAM5	3	177	68

Fig. 9a Relative tumor:normal fold changes in paired tumor/normal gastric samples

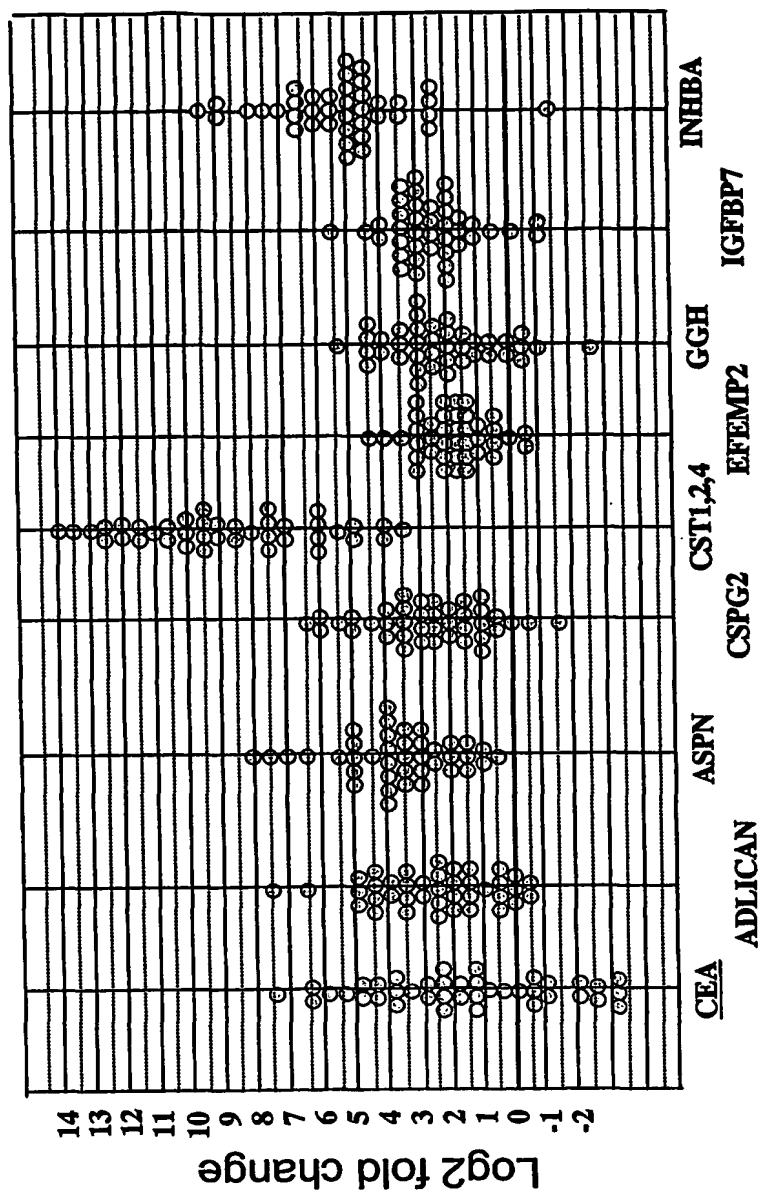


Fig. 9b

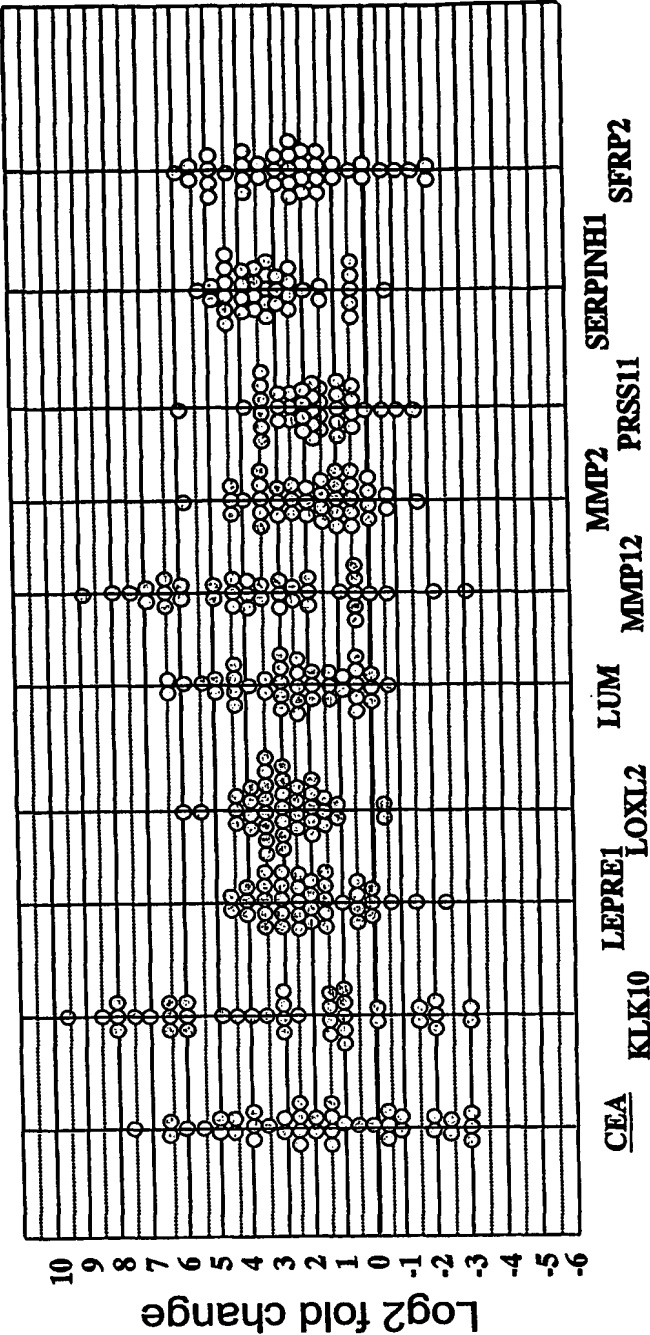


Fig. 9c

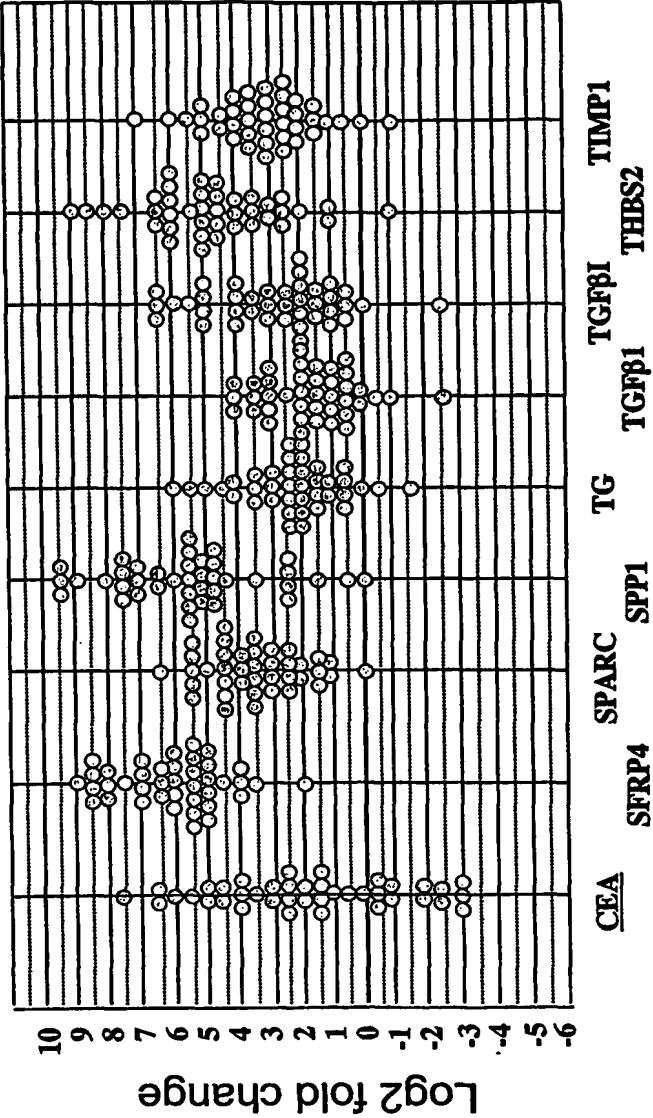


Fig. 9d

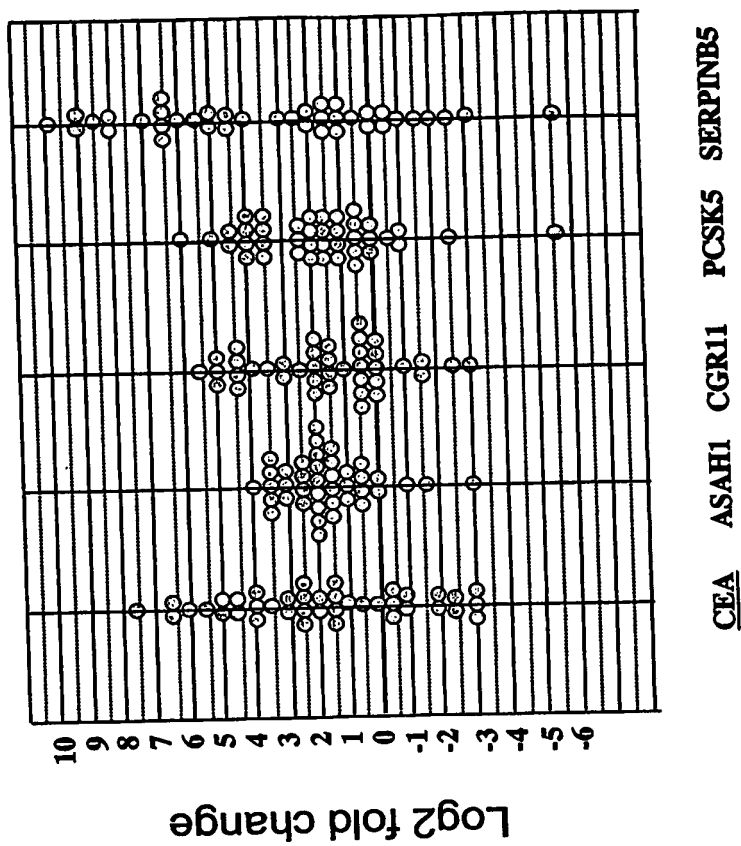


Fig. 10a adlcan

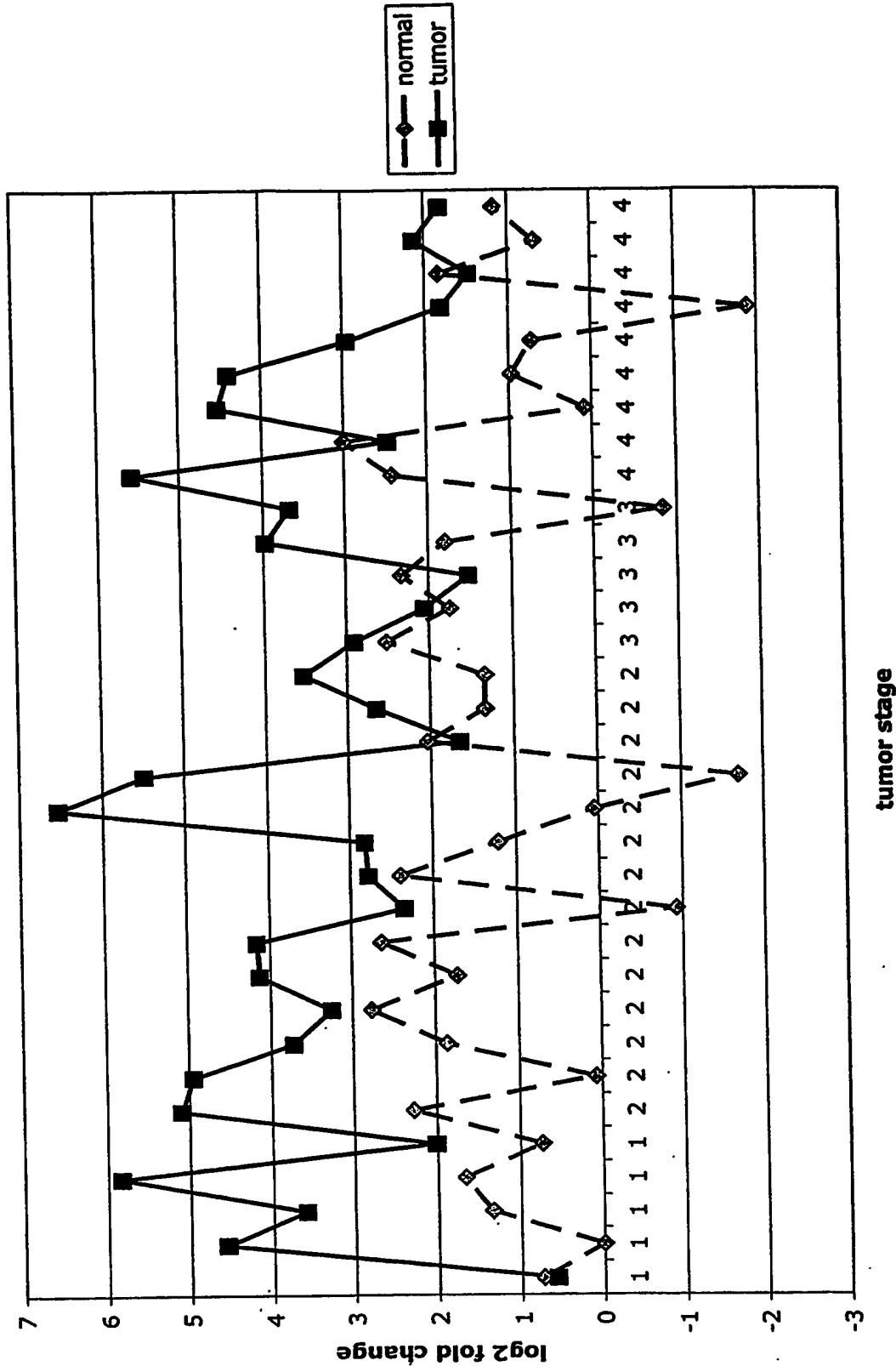


Fig. 10b ASPN

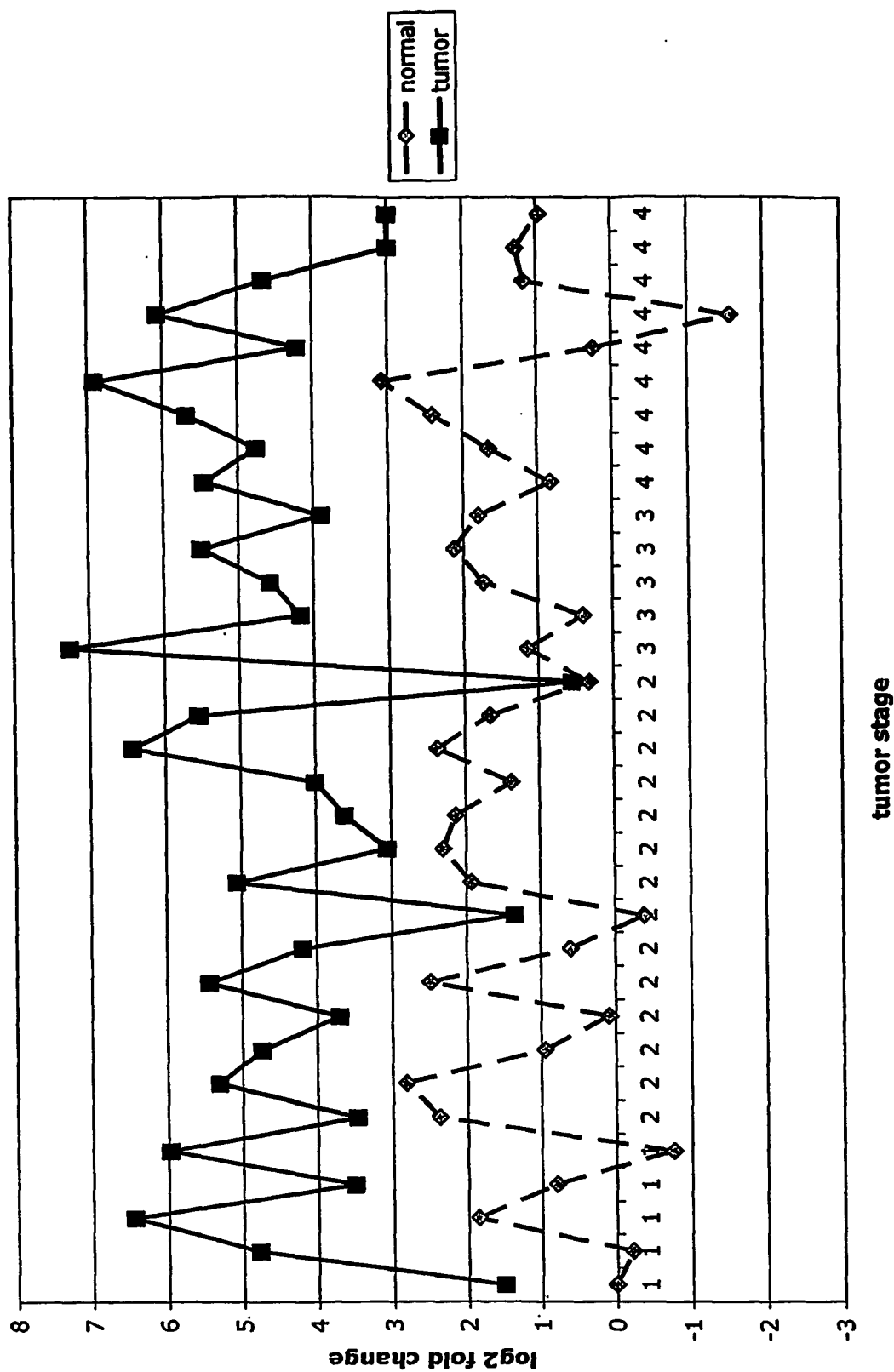


Fig. 10c CSPG2

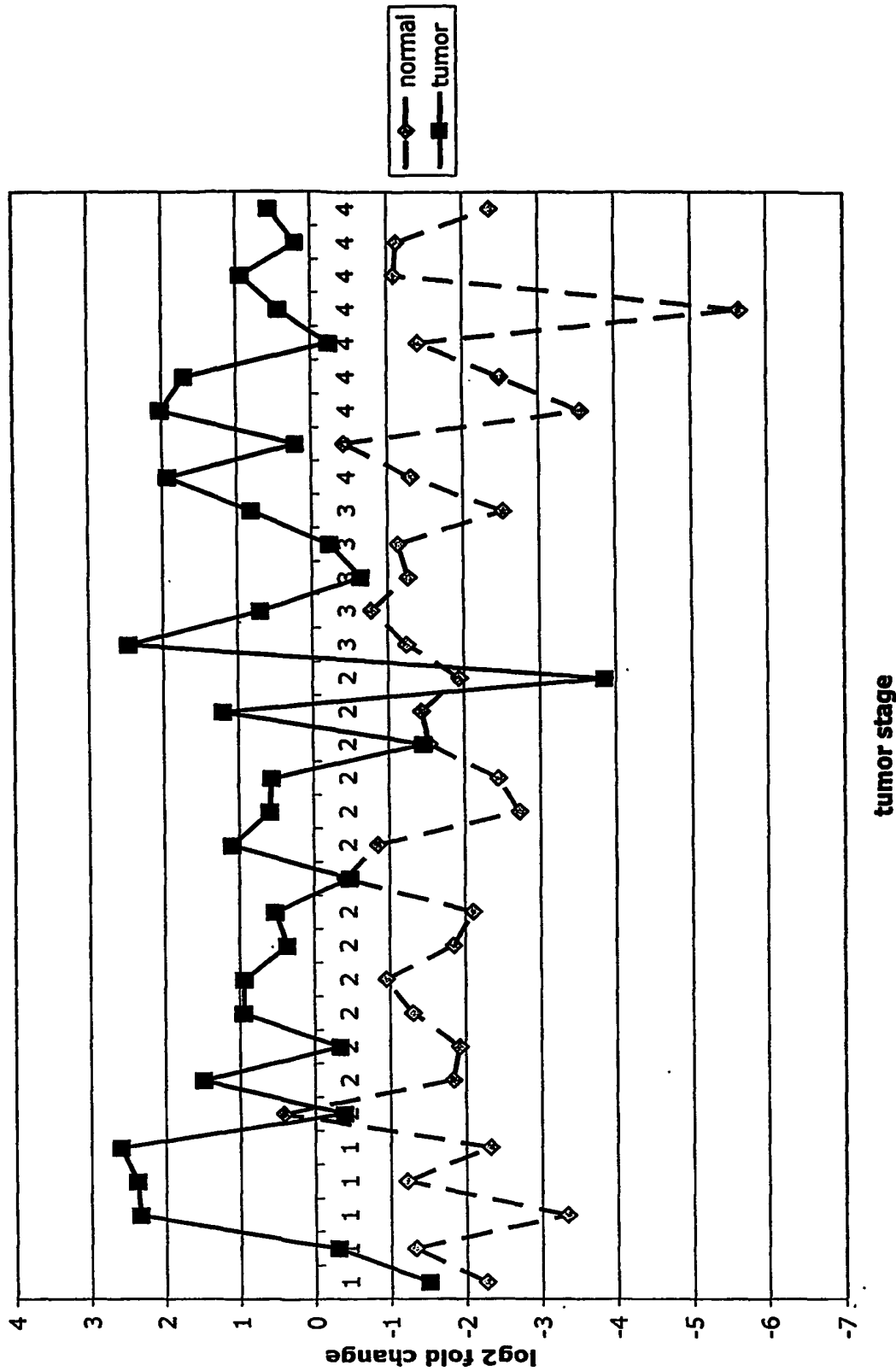


Fig. 10d CST1,2,4

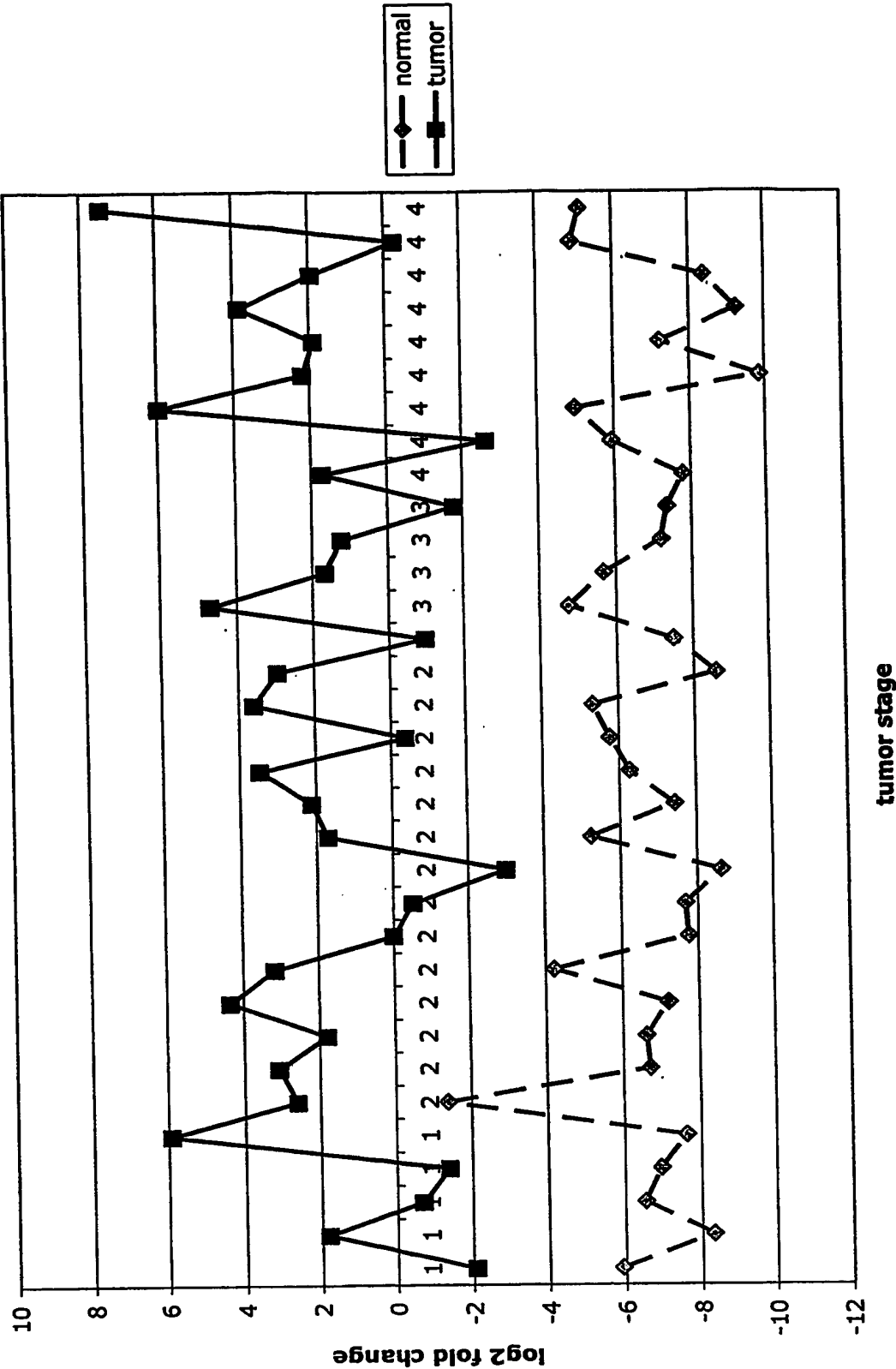


Fig. 10e EFEMP2

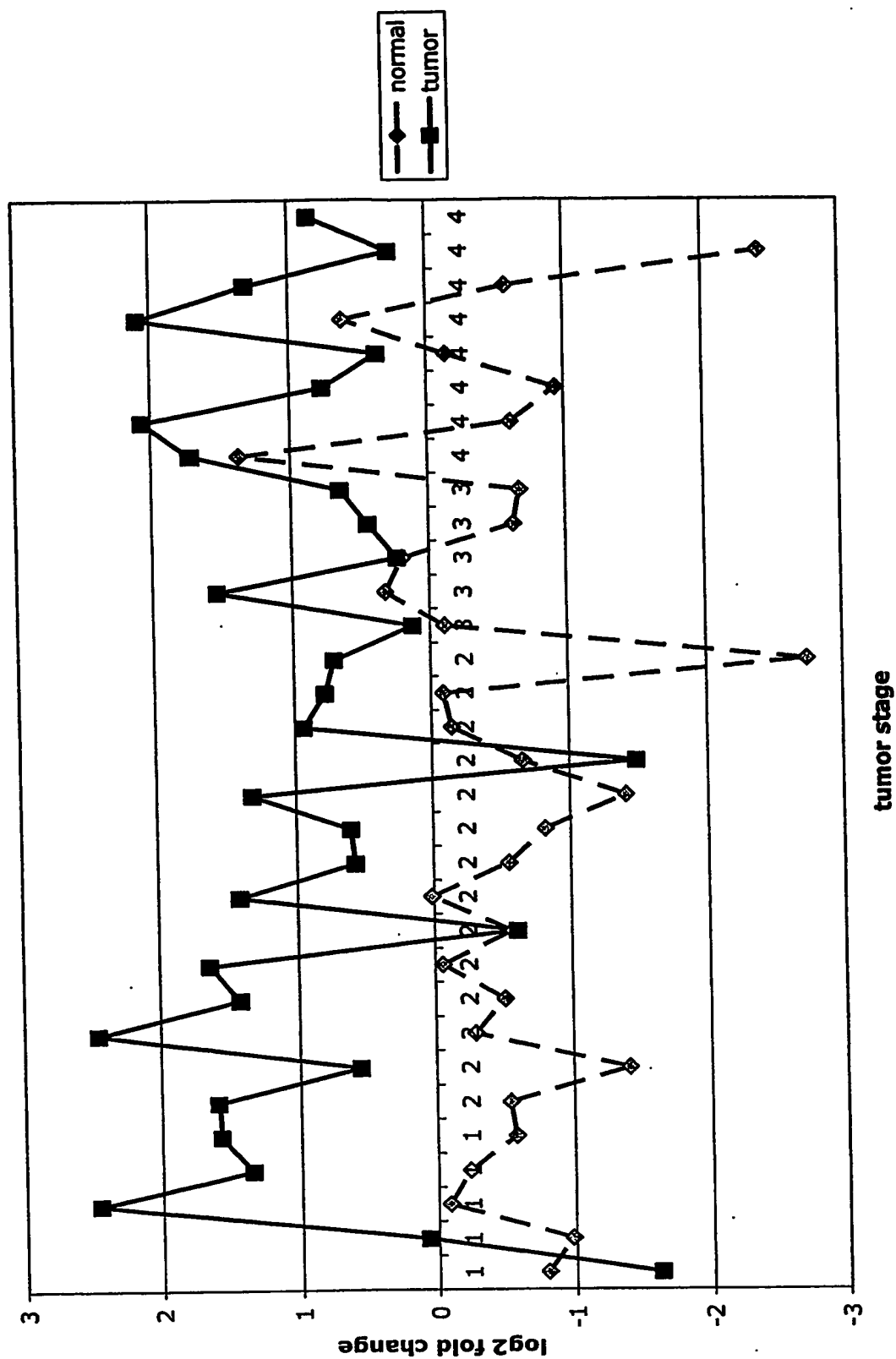
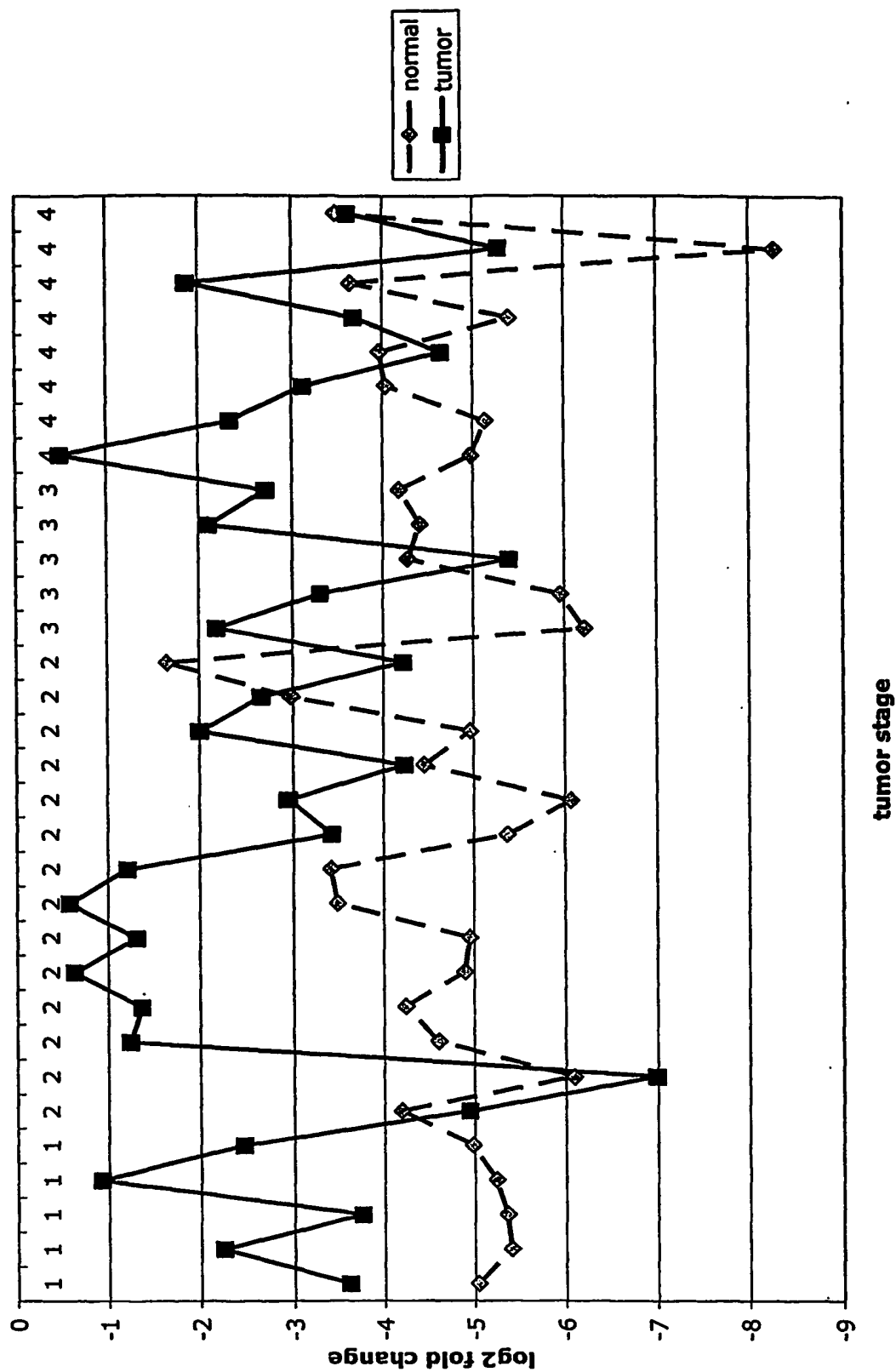


Fig. 10f GGH



46/104

Fig. 10g INHBA

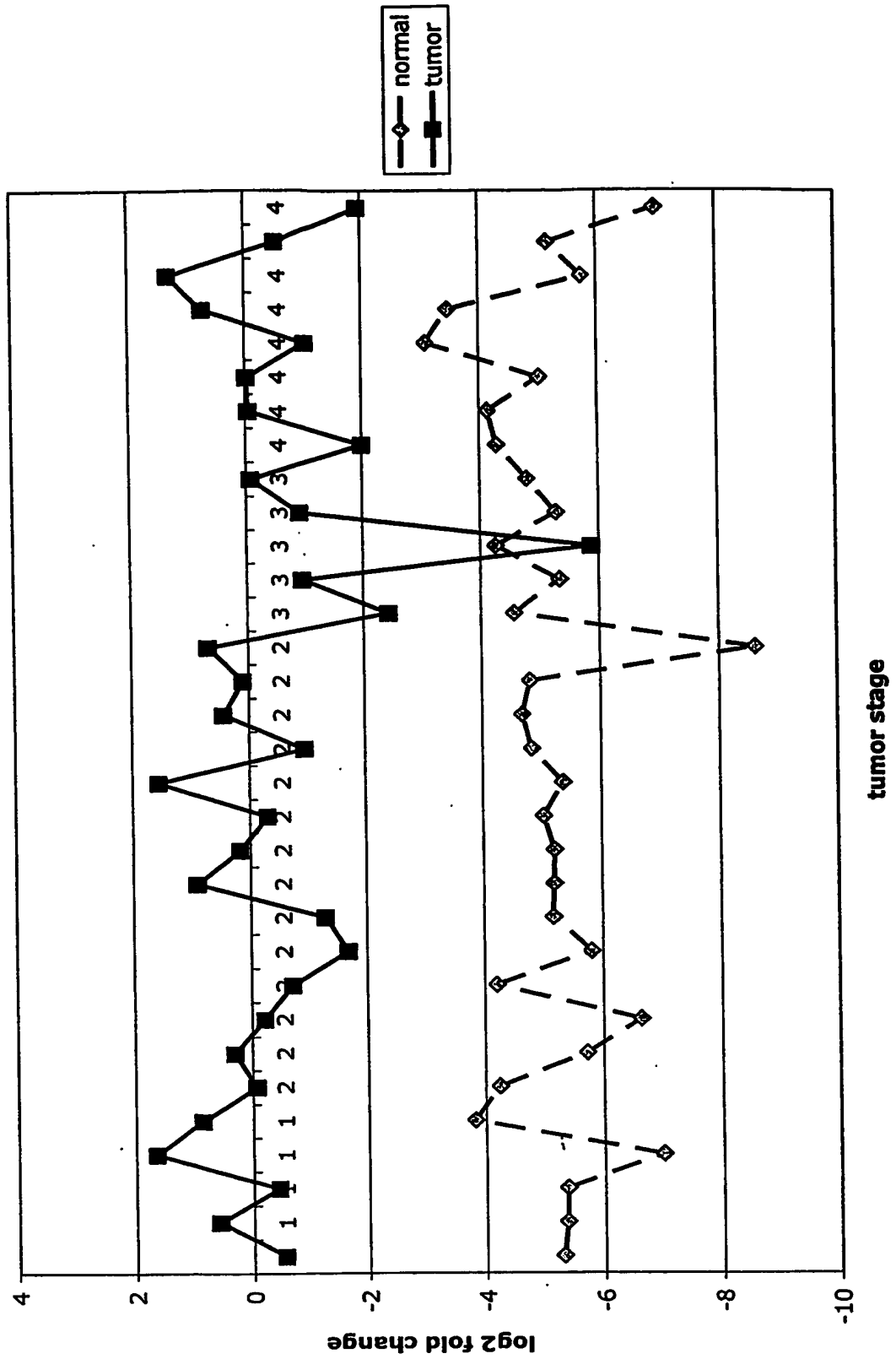


Fig. 10h IGFBP7

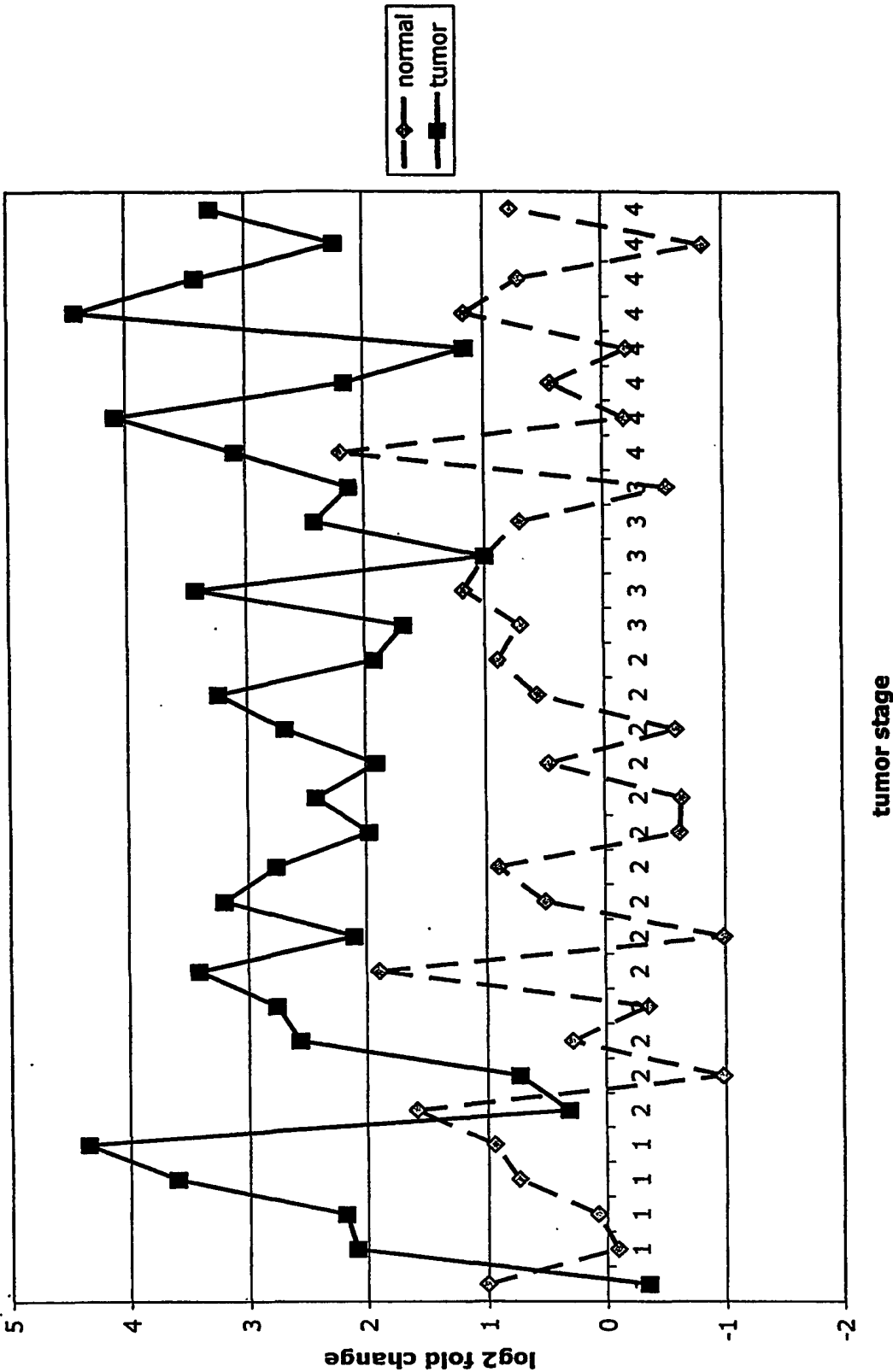
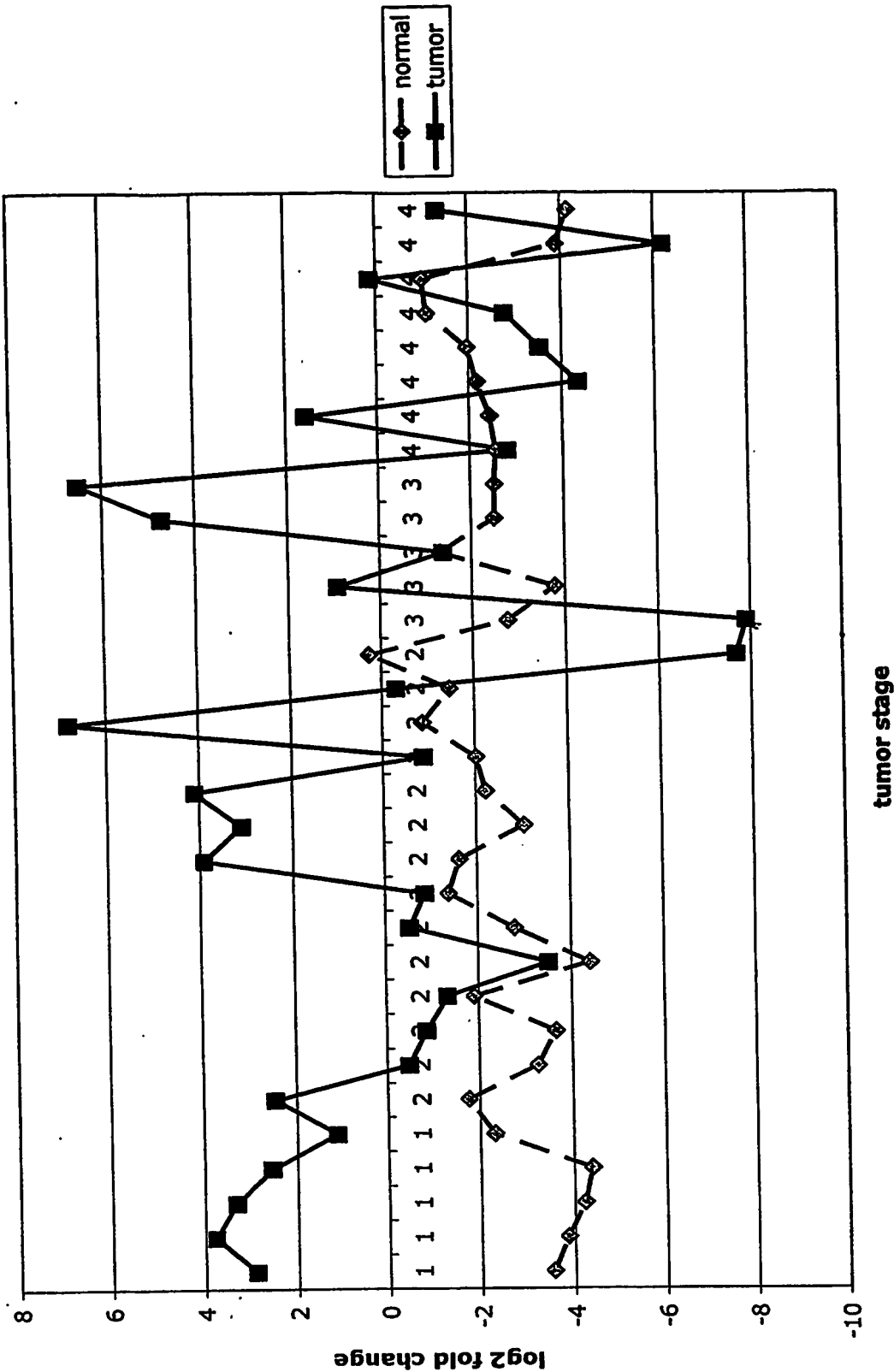


Fig. 10i KLK10



49/104

Fig. 10j LEPRE1

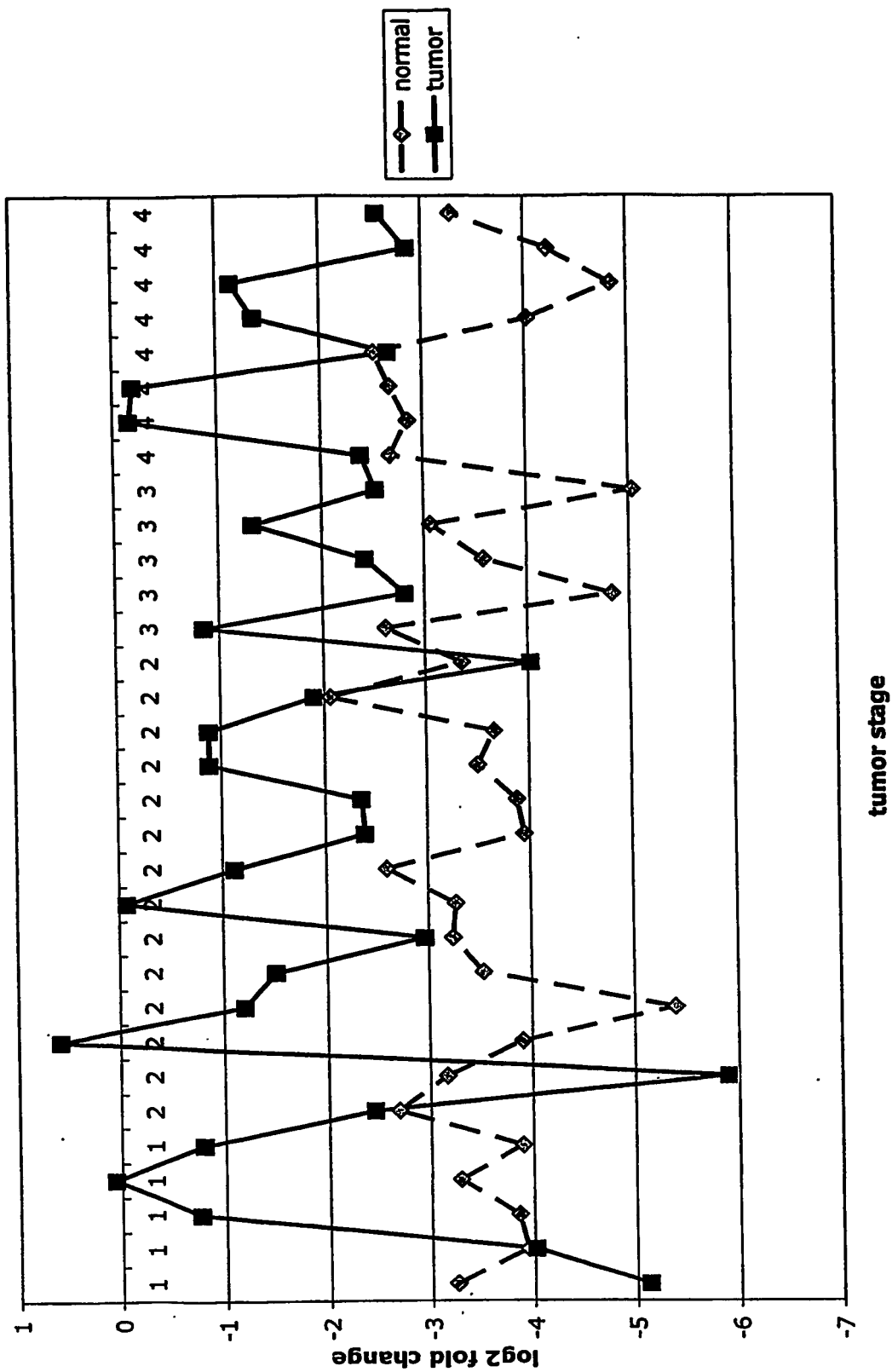


Fig. 10k LUM

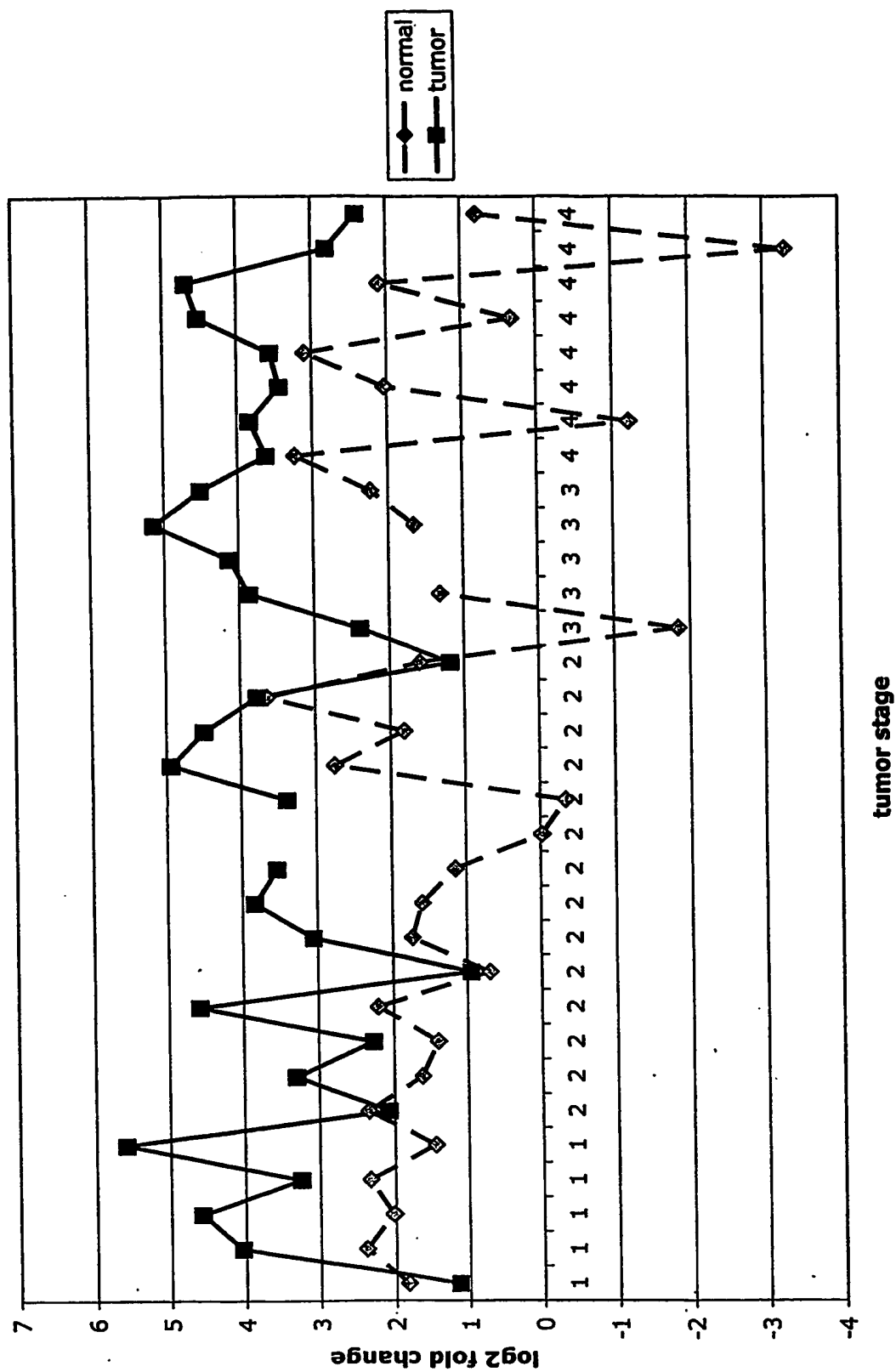


Fig. 10I LOXL2

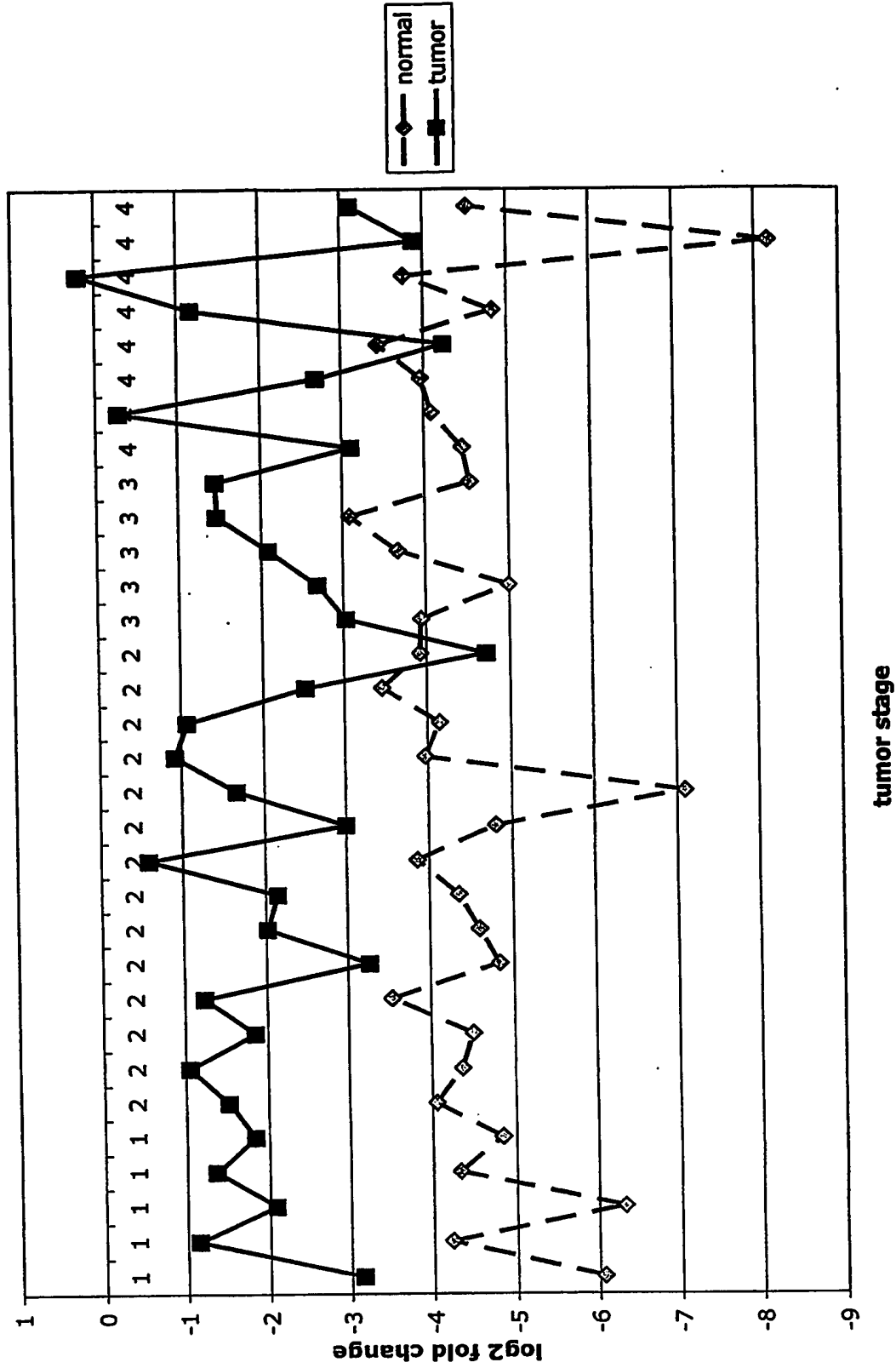


Fig. 10m MMP12

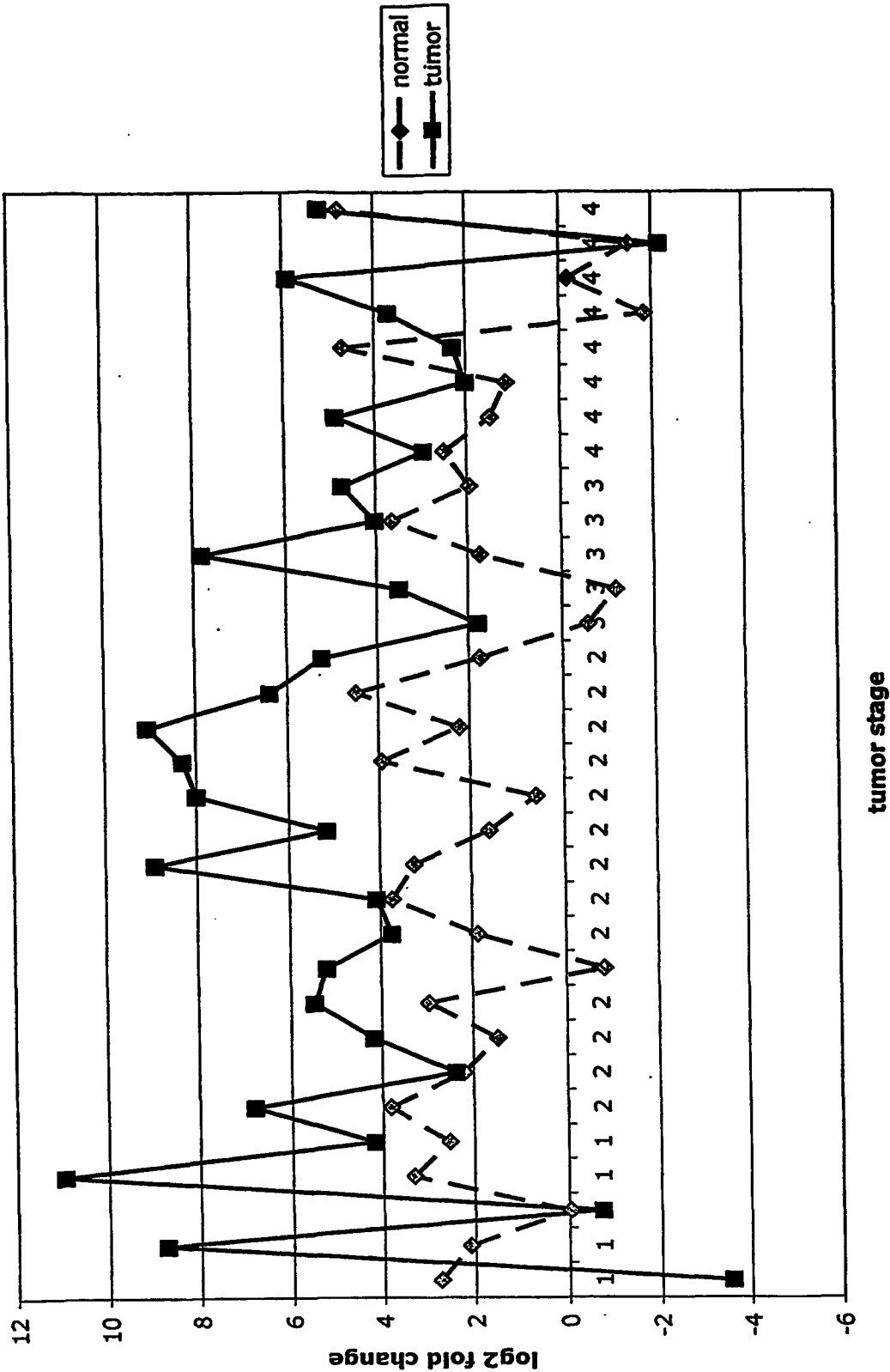


Fig.10n TIMP1

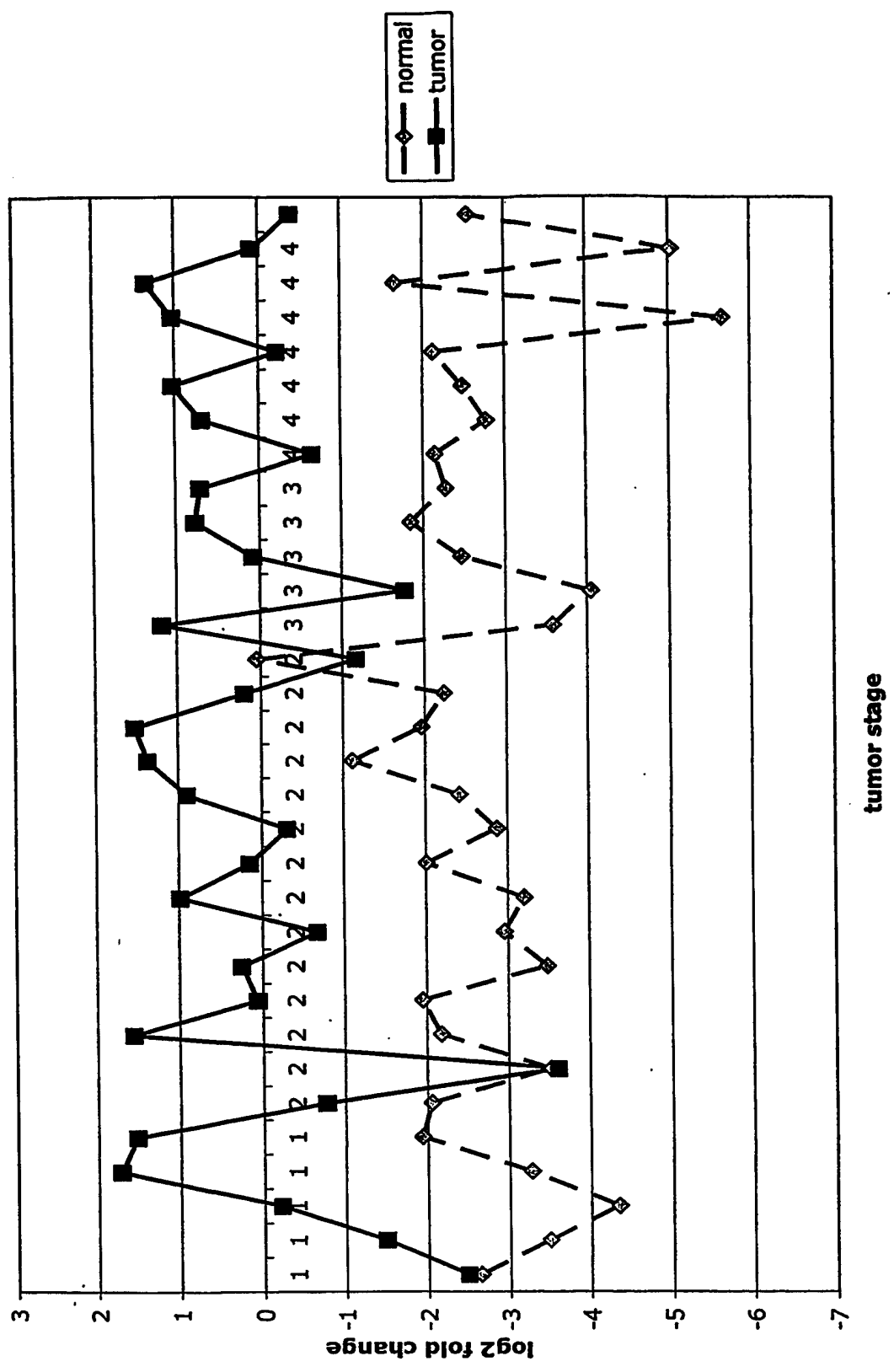


Fig. 10o ASAH1

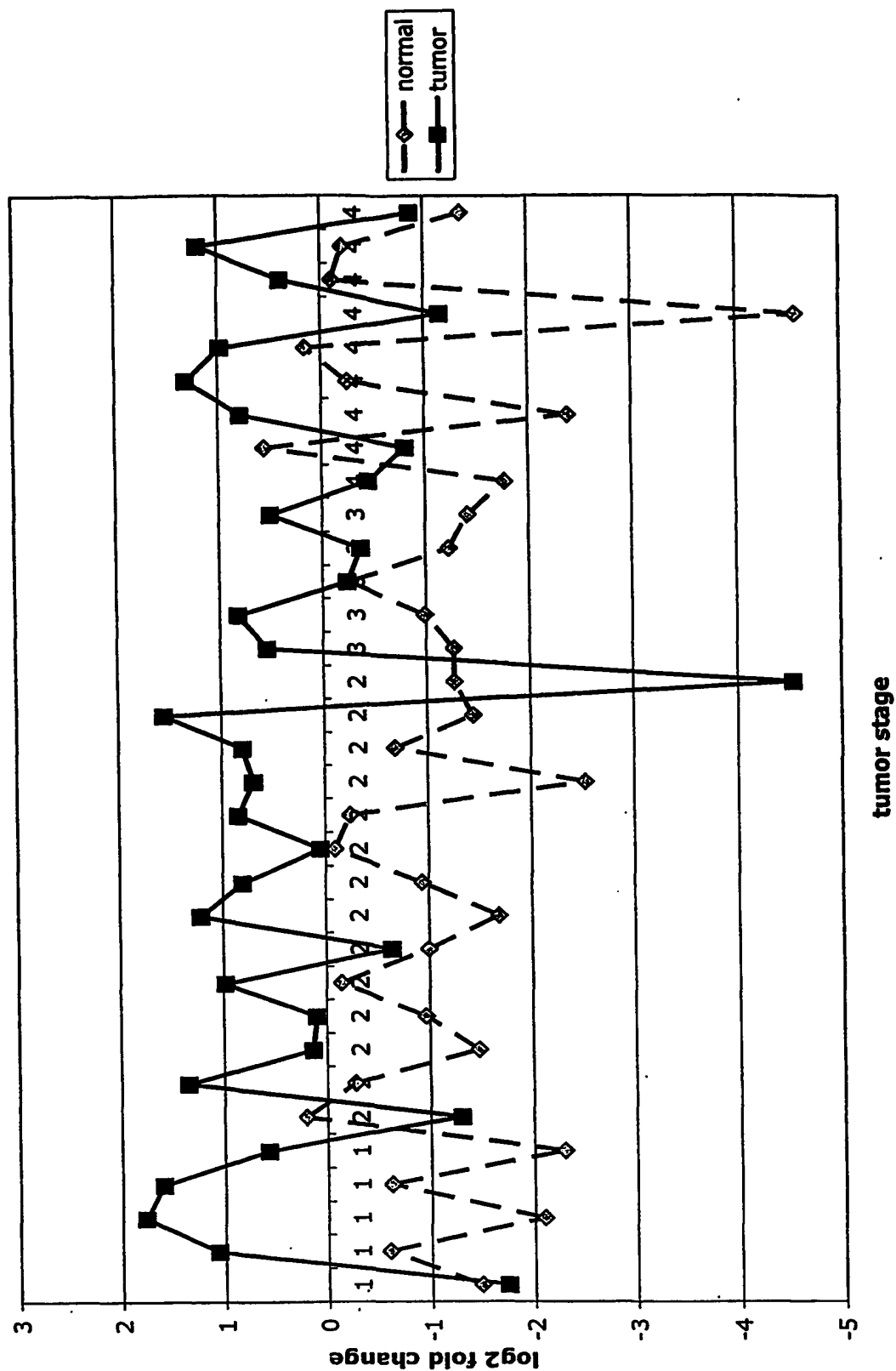


Fig. 10p SPP1

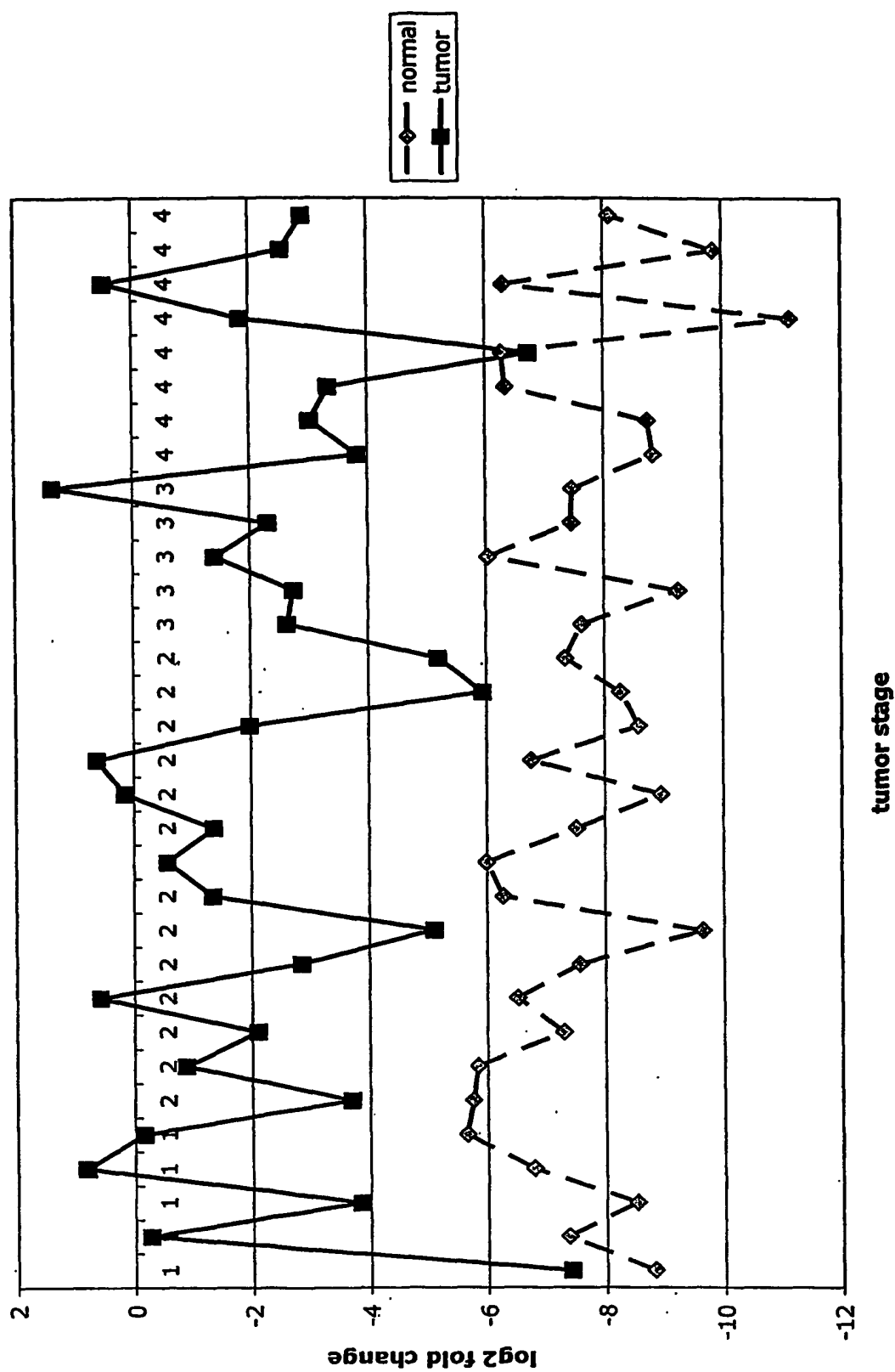


Fig. 10q SFRP2

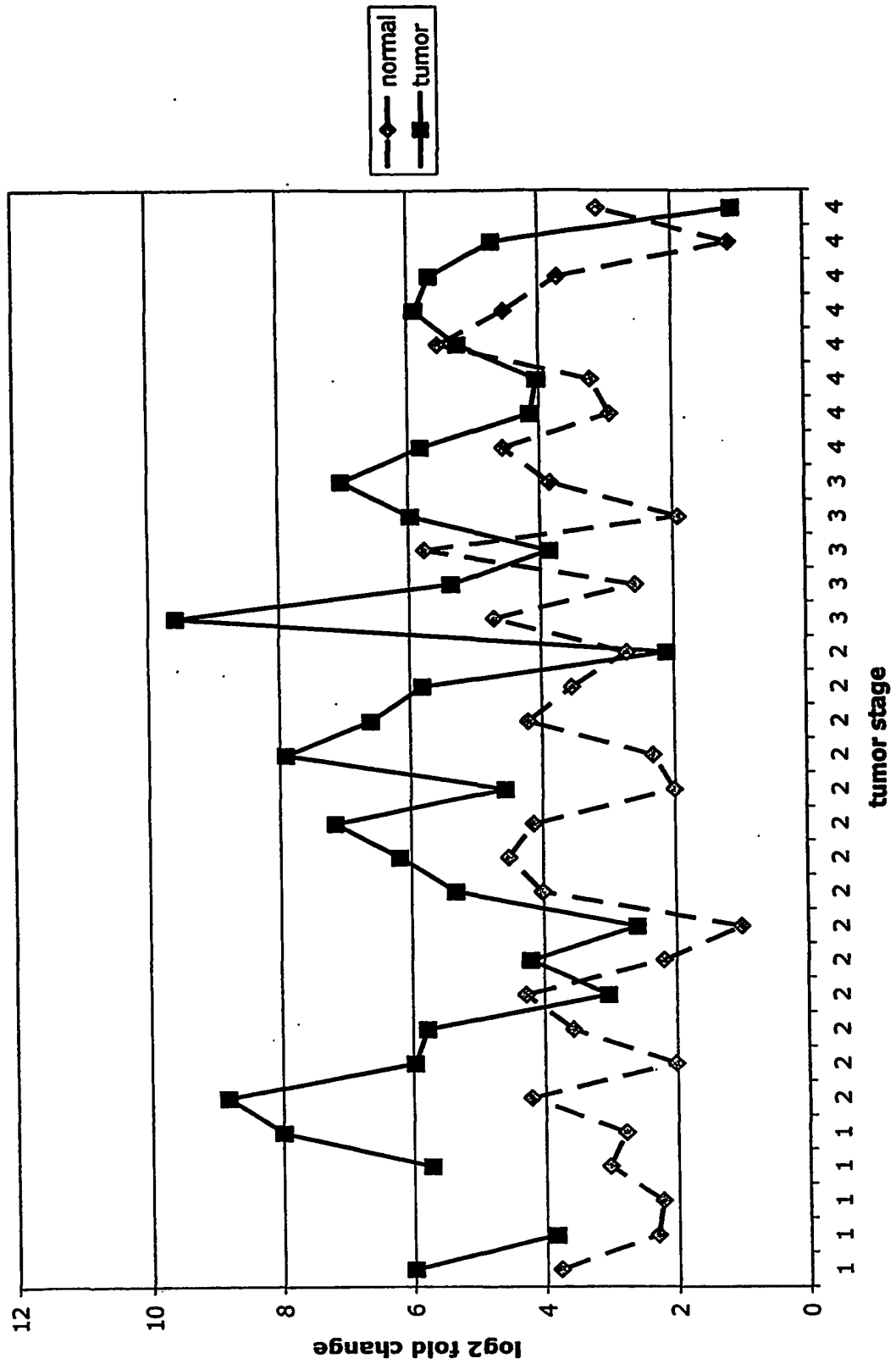


Fig. 10r SFRP4

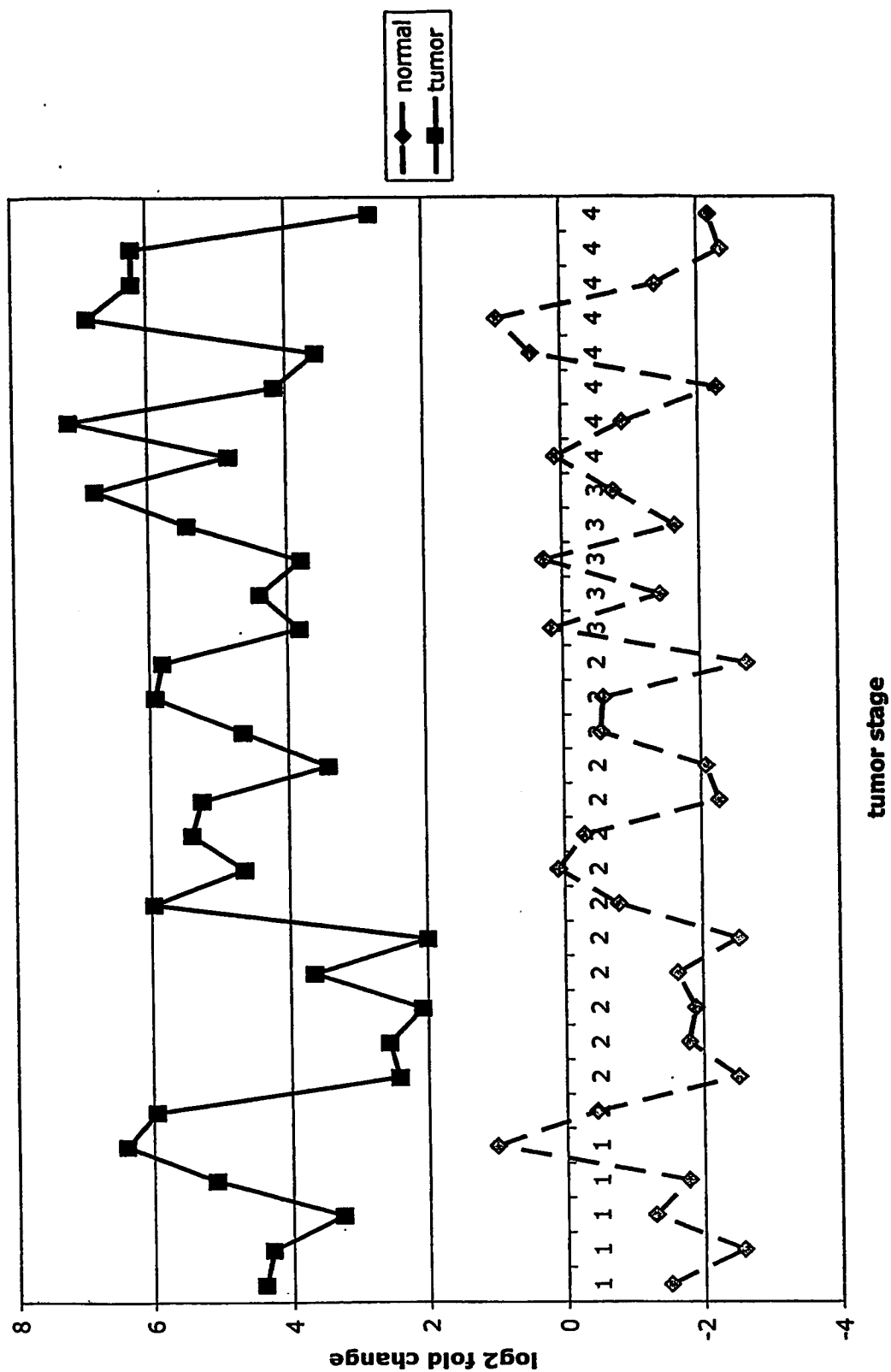


Fig. 10s SPARC

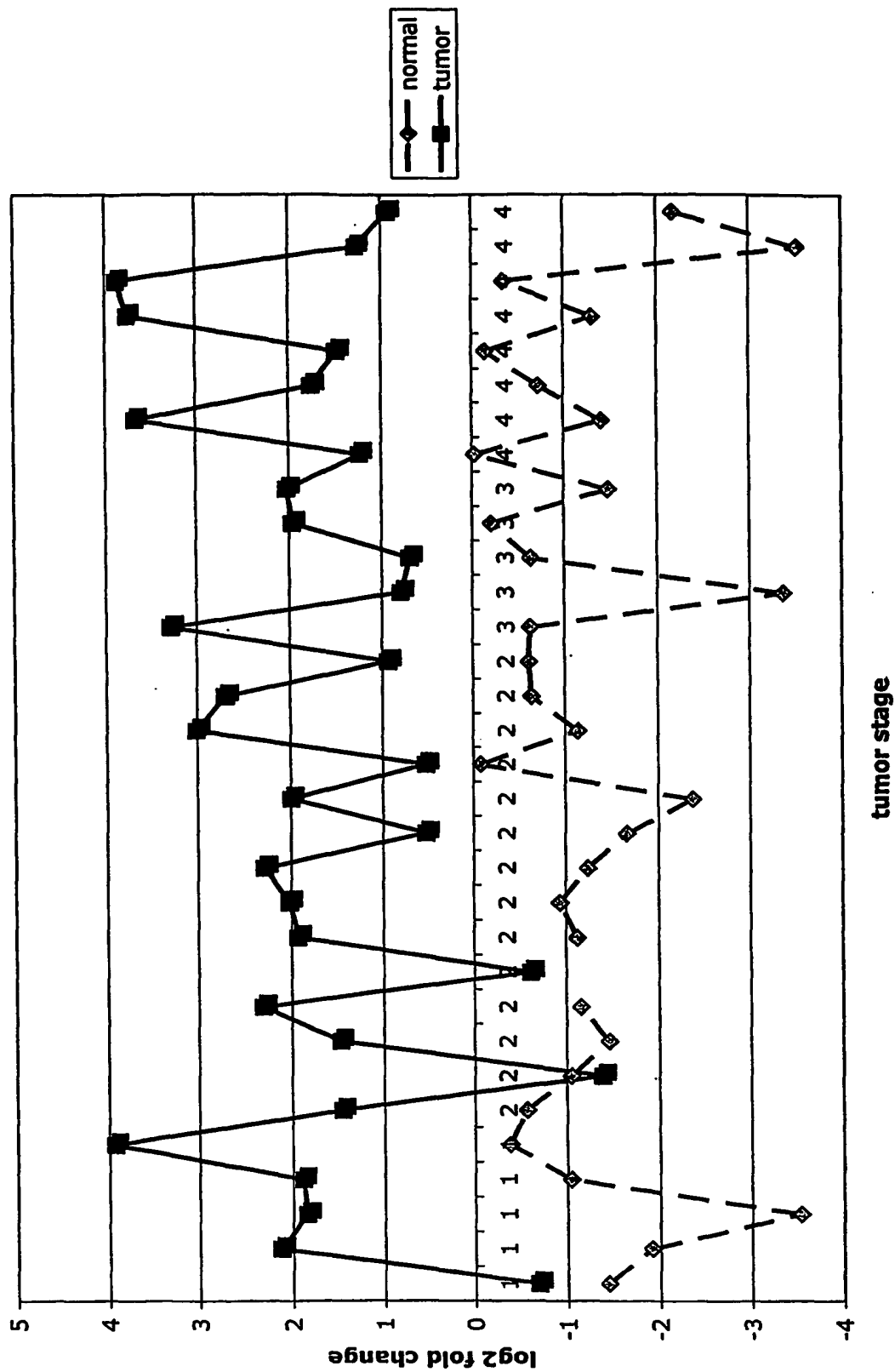


Fig. 10t PRSS11

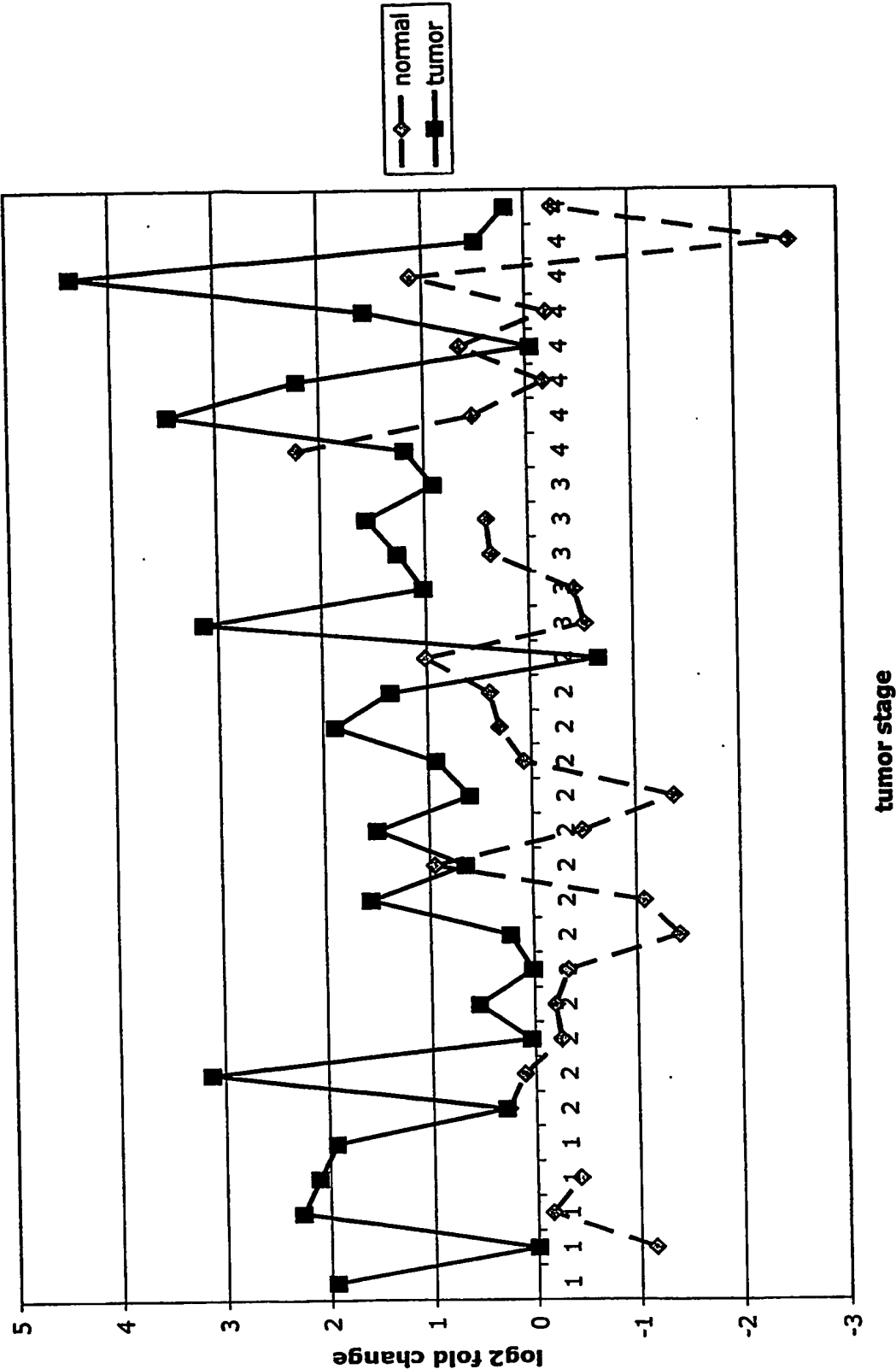


Fig. 10u THBS2

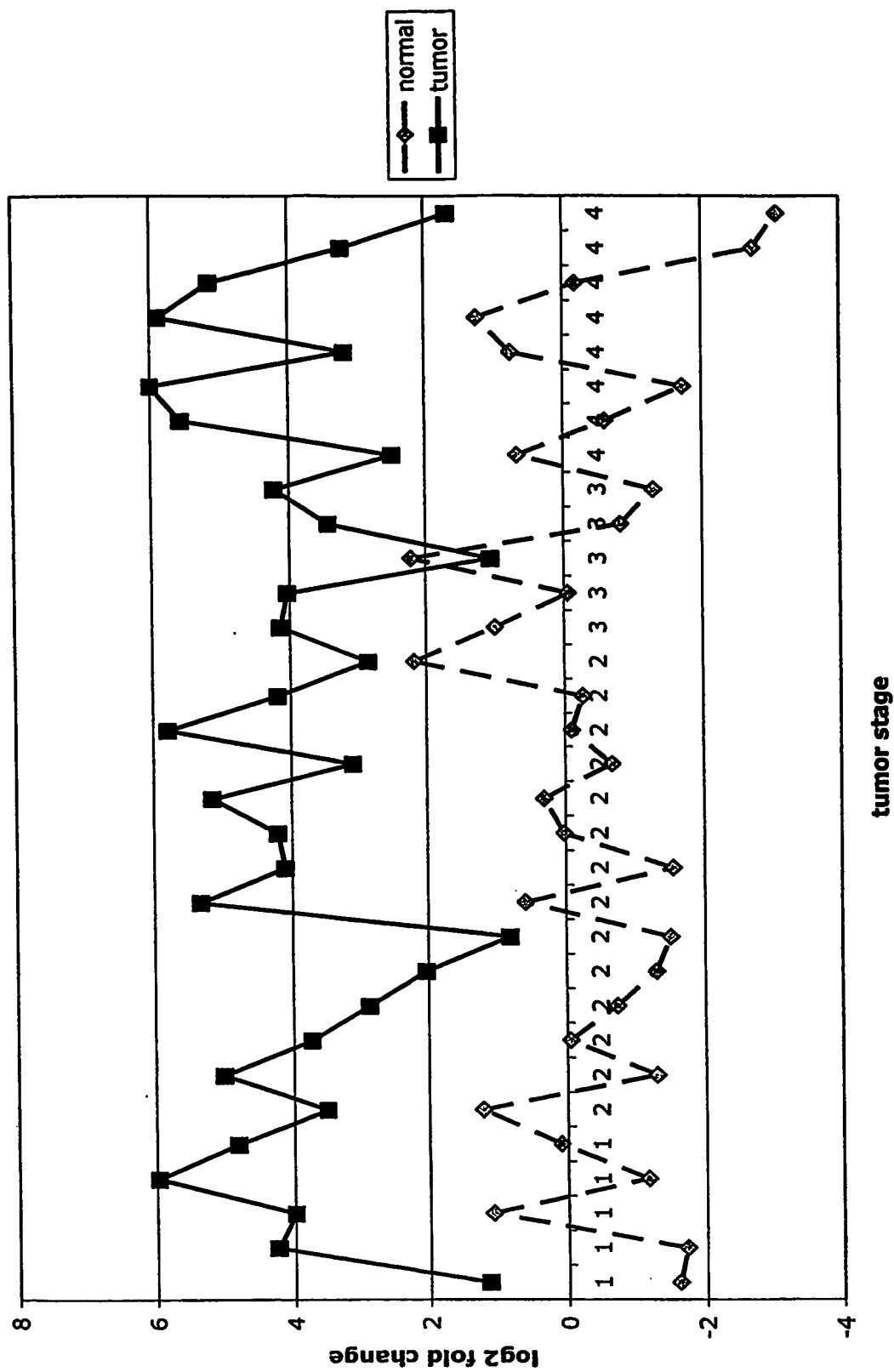


Fig. 10v TG

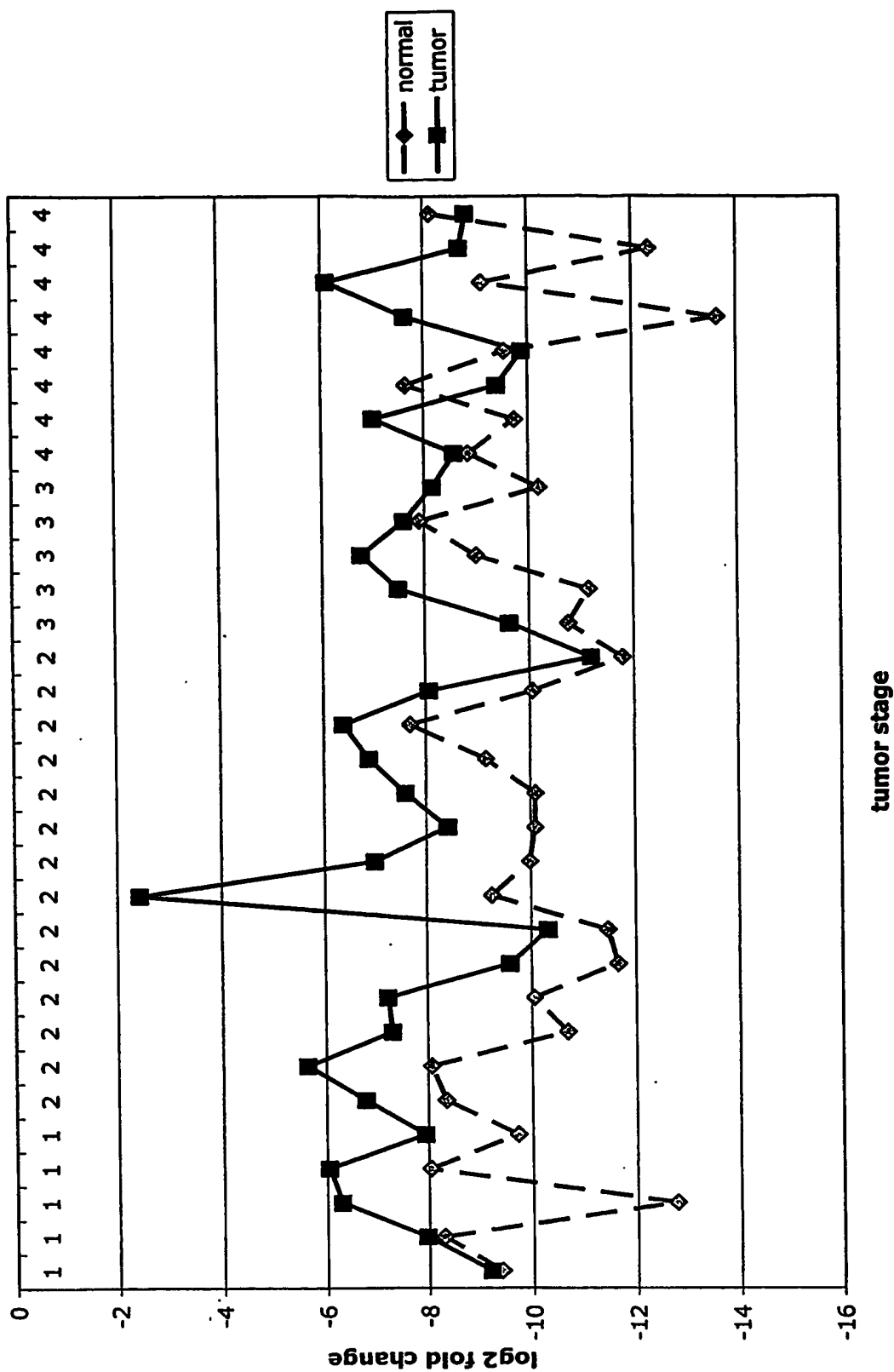
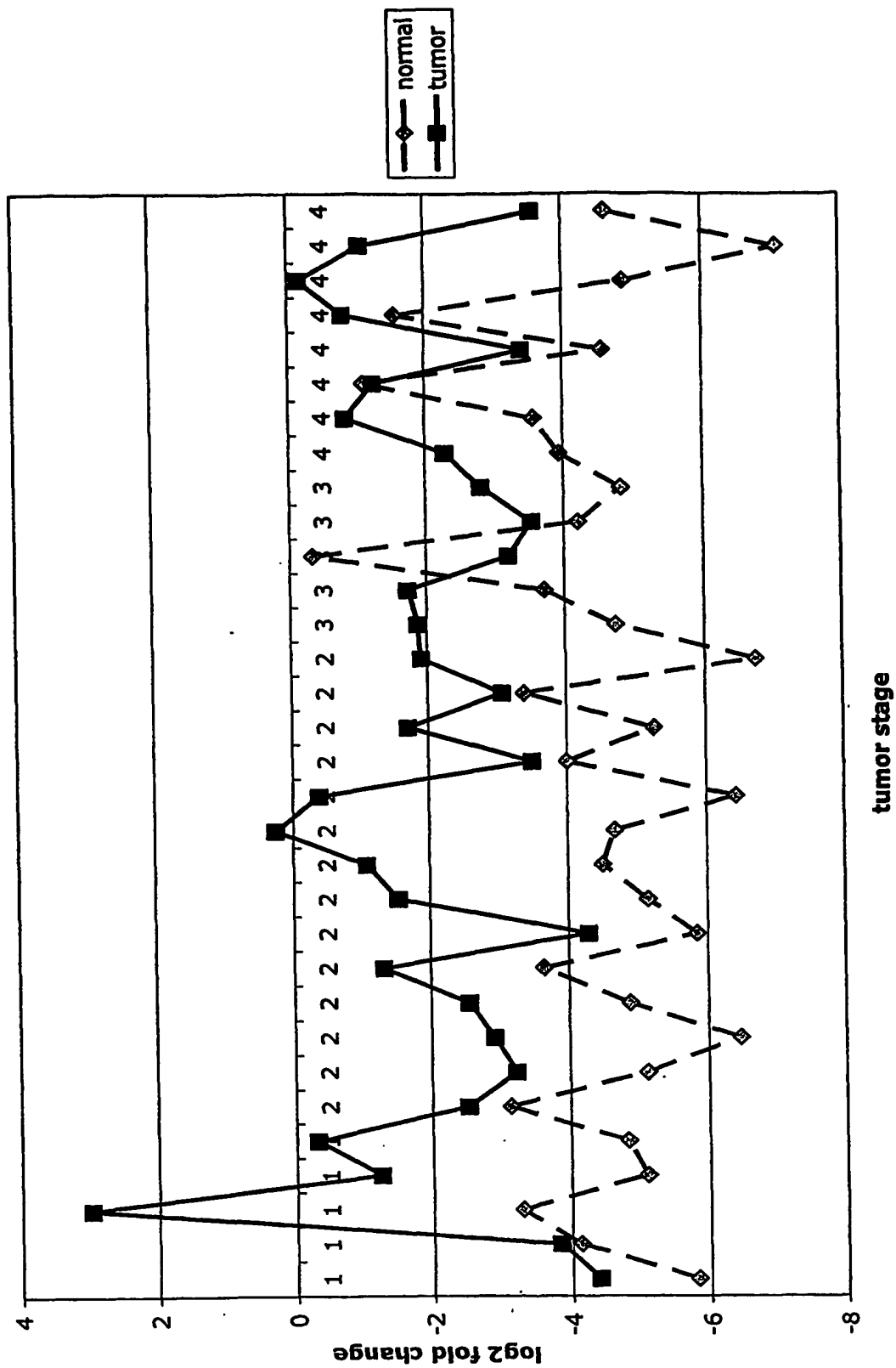


Fig. 10w TGFBI



63/104

Fig. 10x CGR11

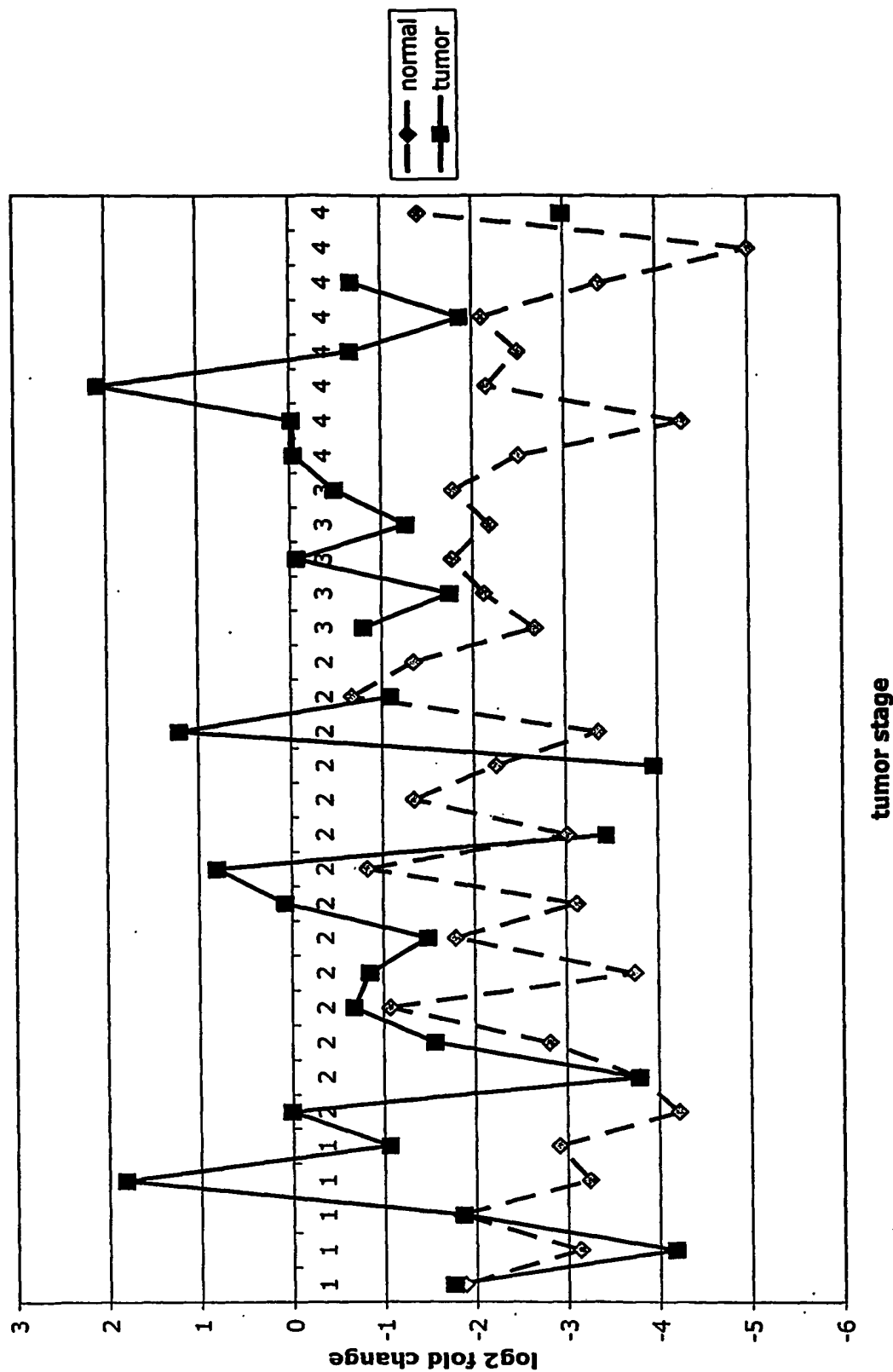


Fig. 10y SERPINH1

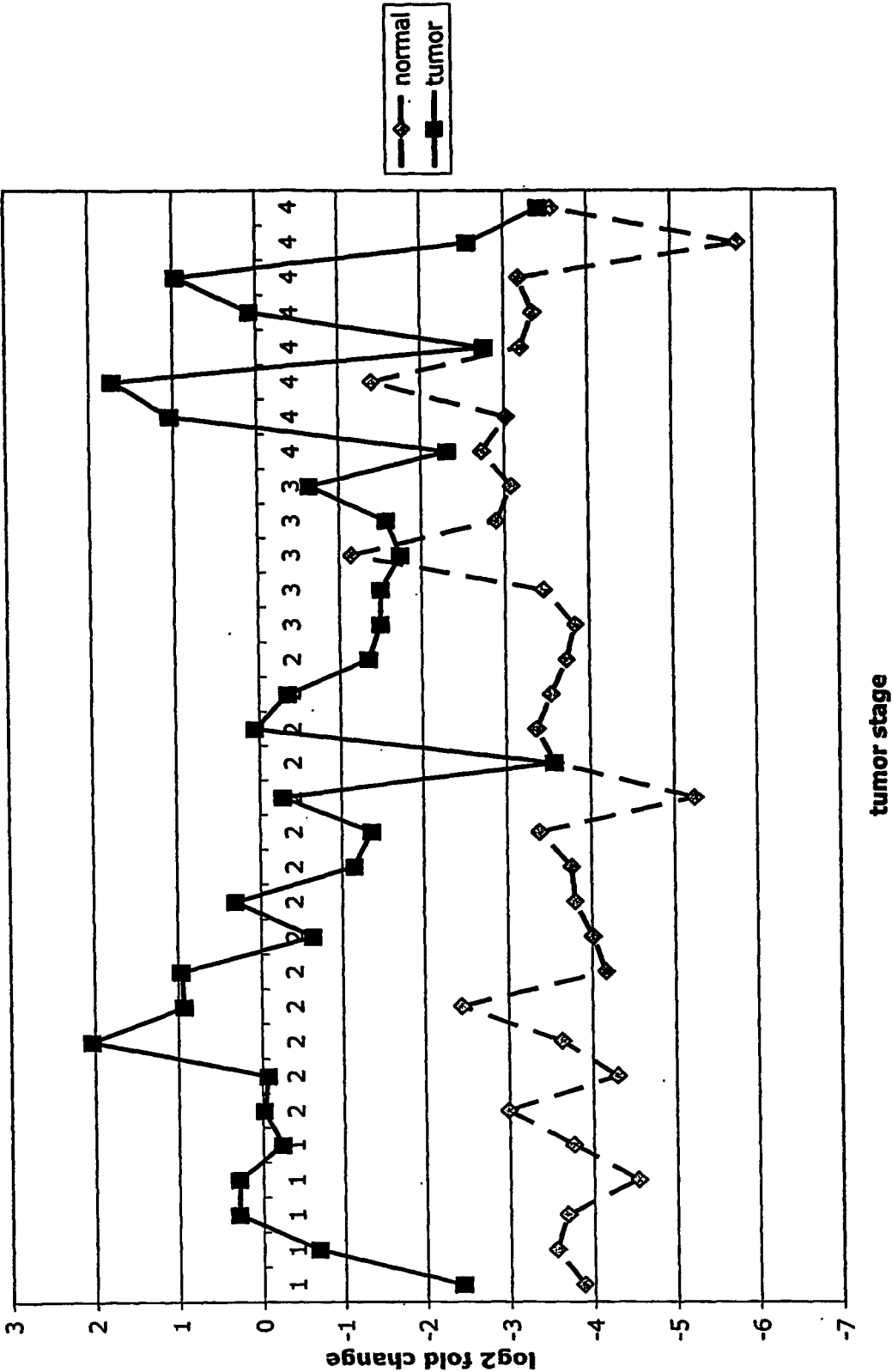


Fig. 10z MMP2

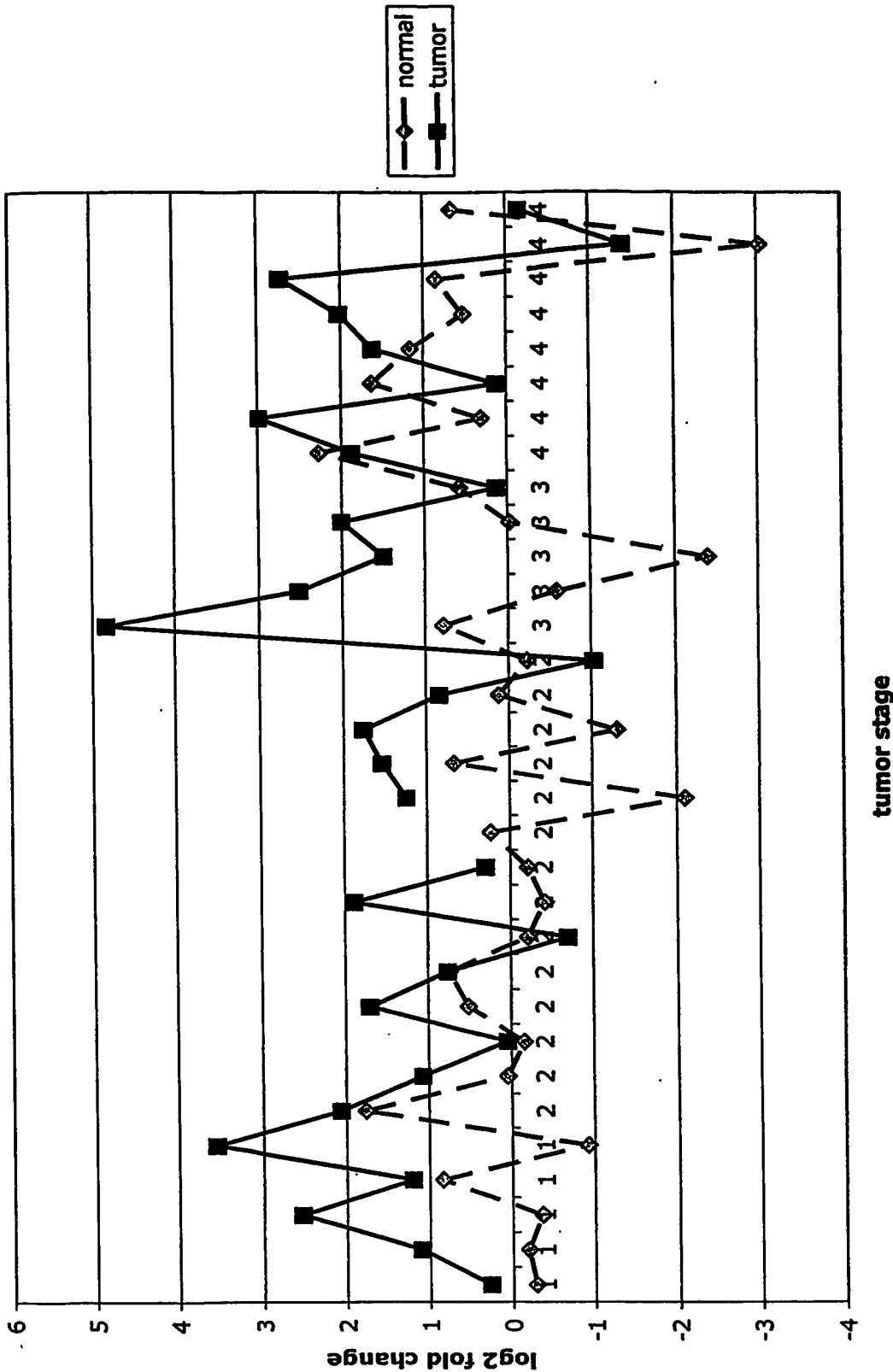
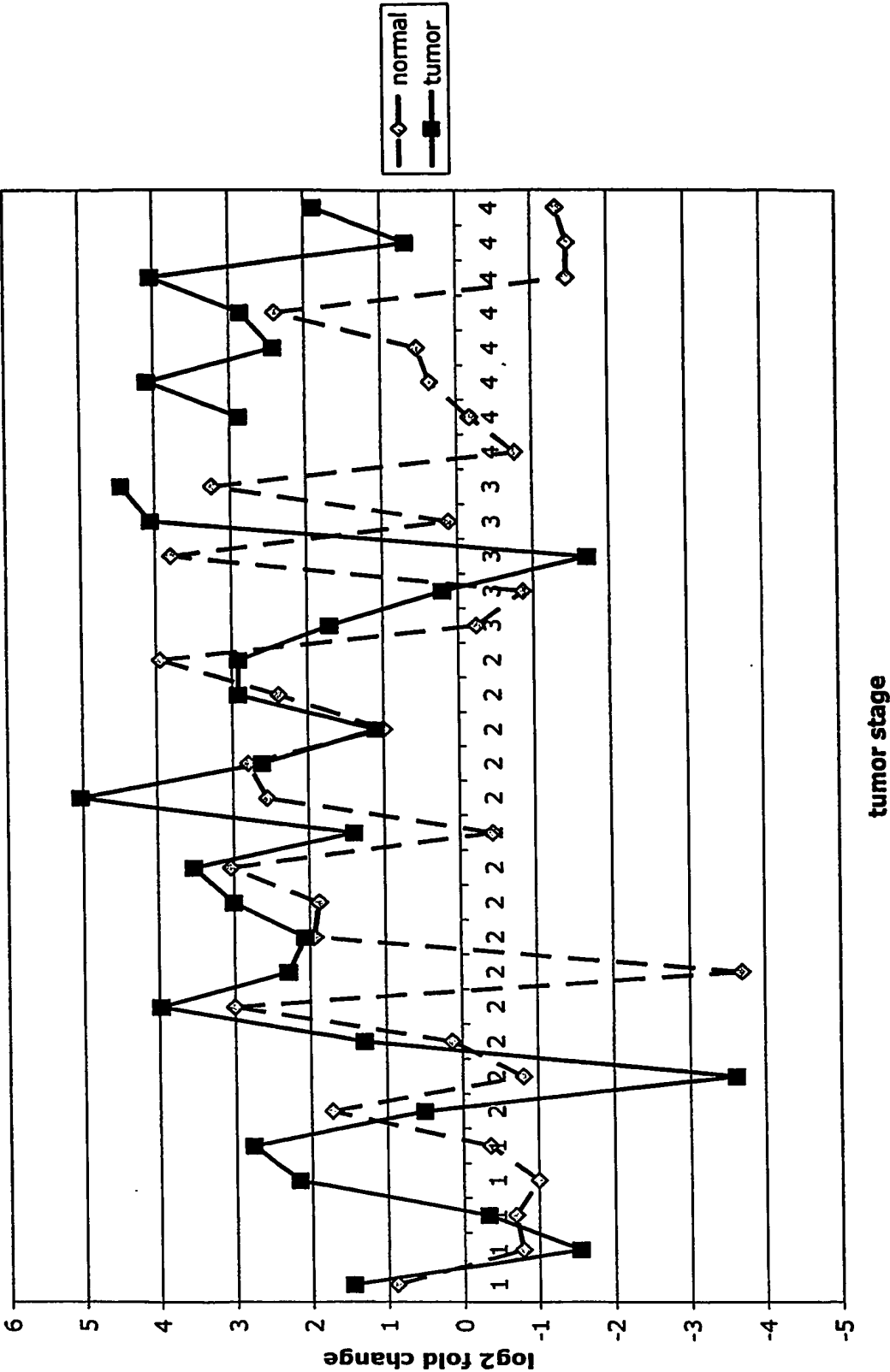


Fig. 10aa PCSK5



67/104

Fig. 10ab SERPINB5

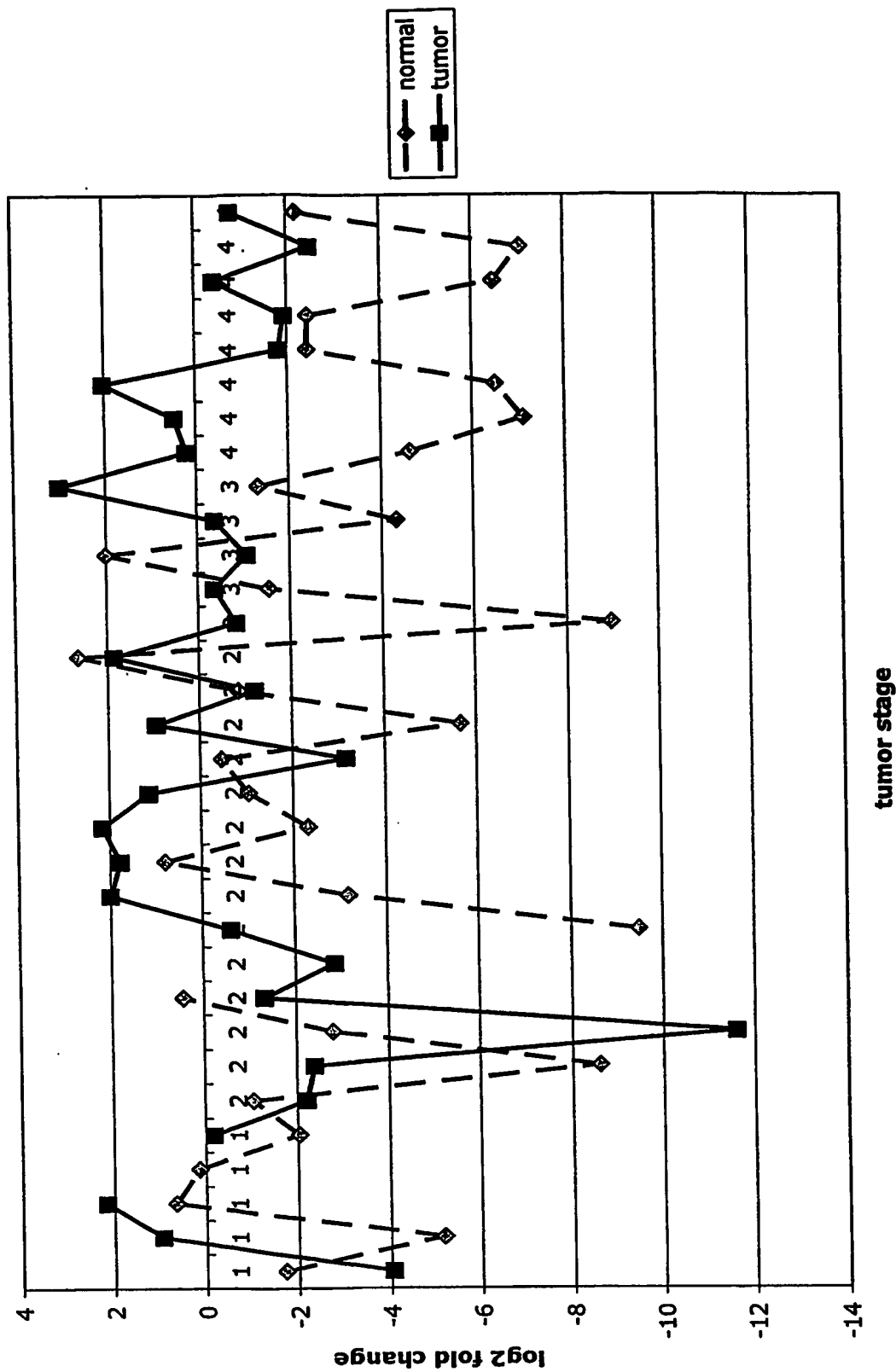


Fig. 10ac TGFb1

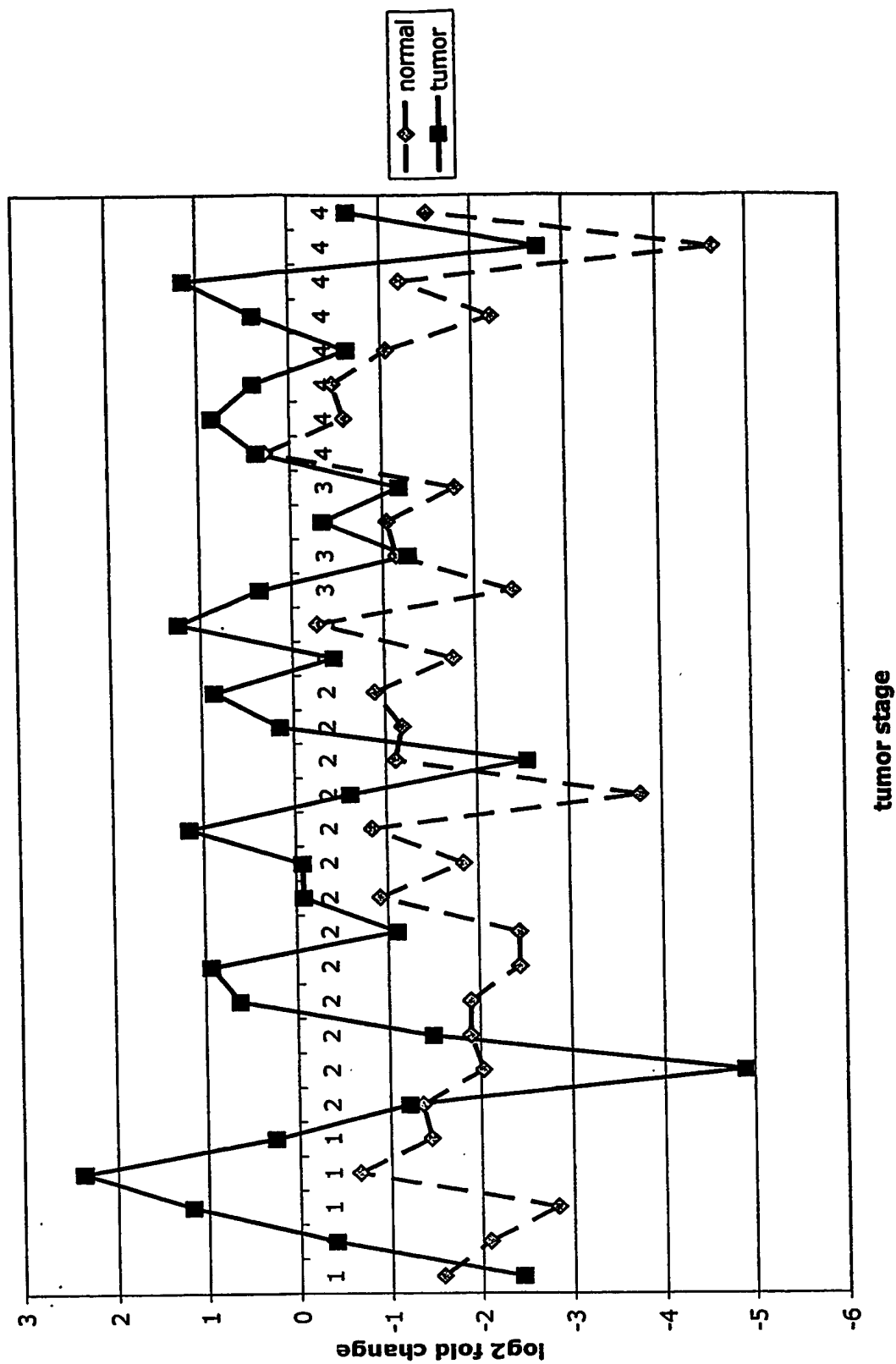
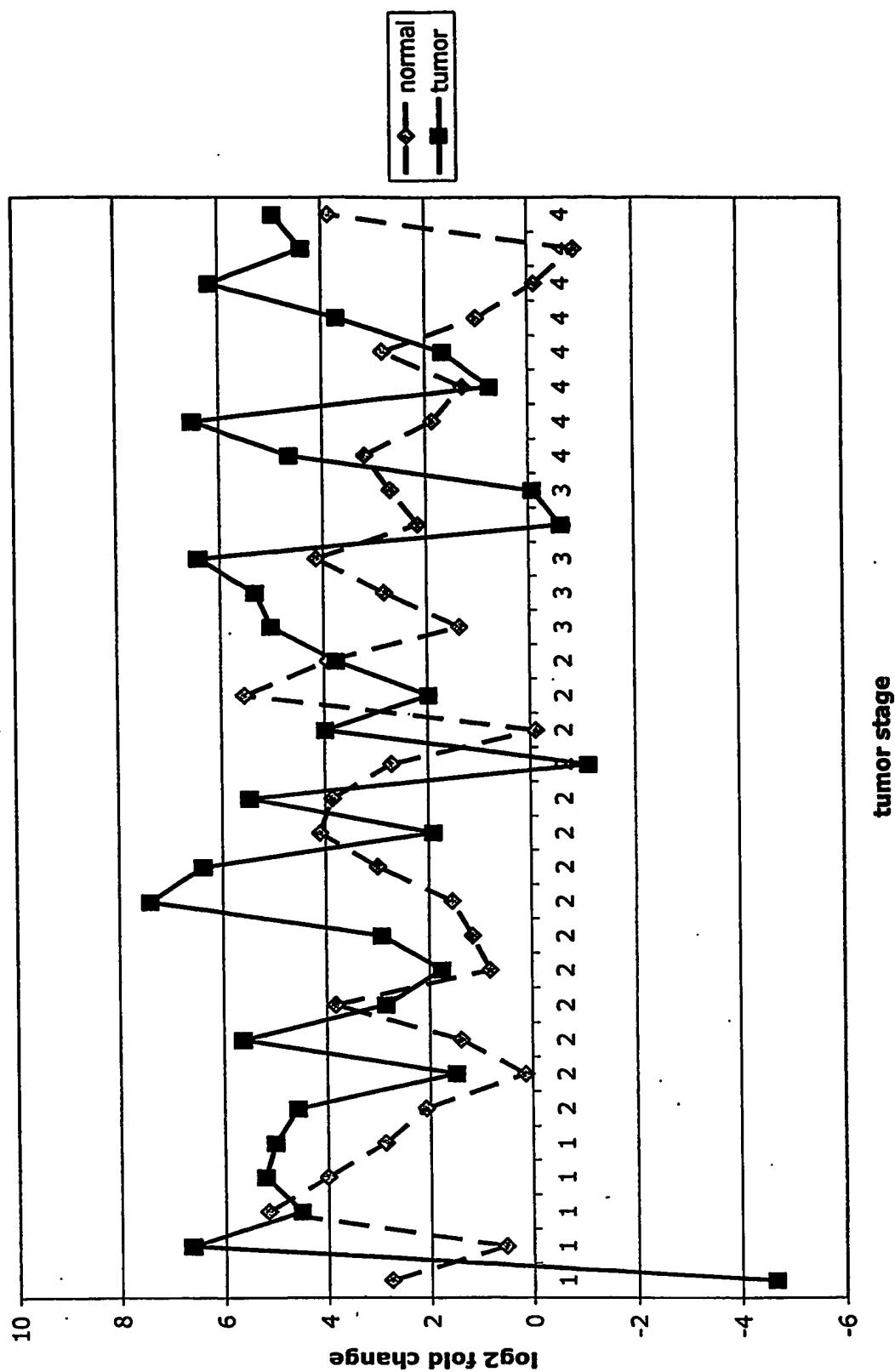


Fig. 10ad CEA



70/104

Fig. 11a Adican

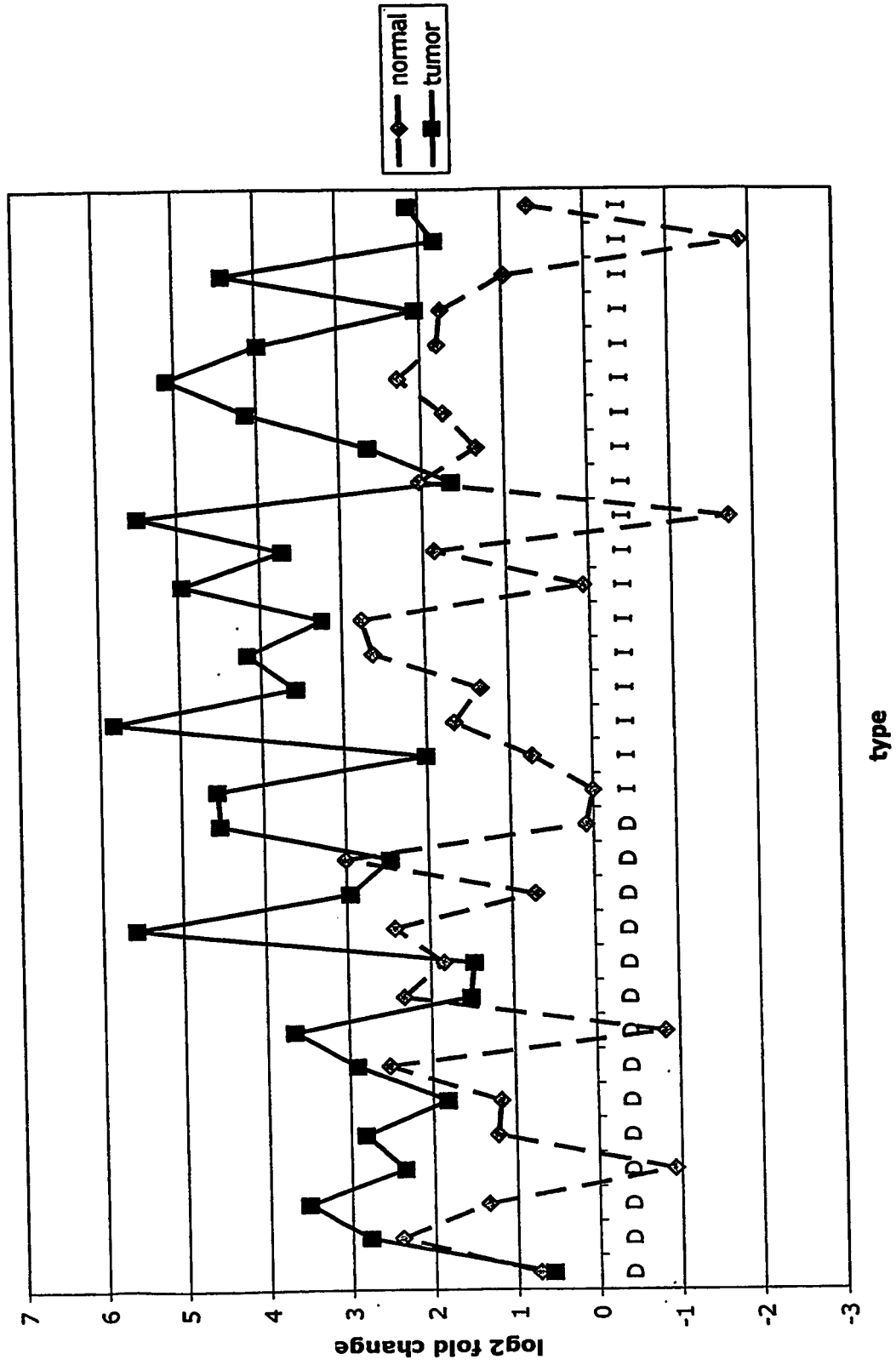


Fig. 11b ASPN

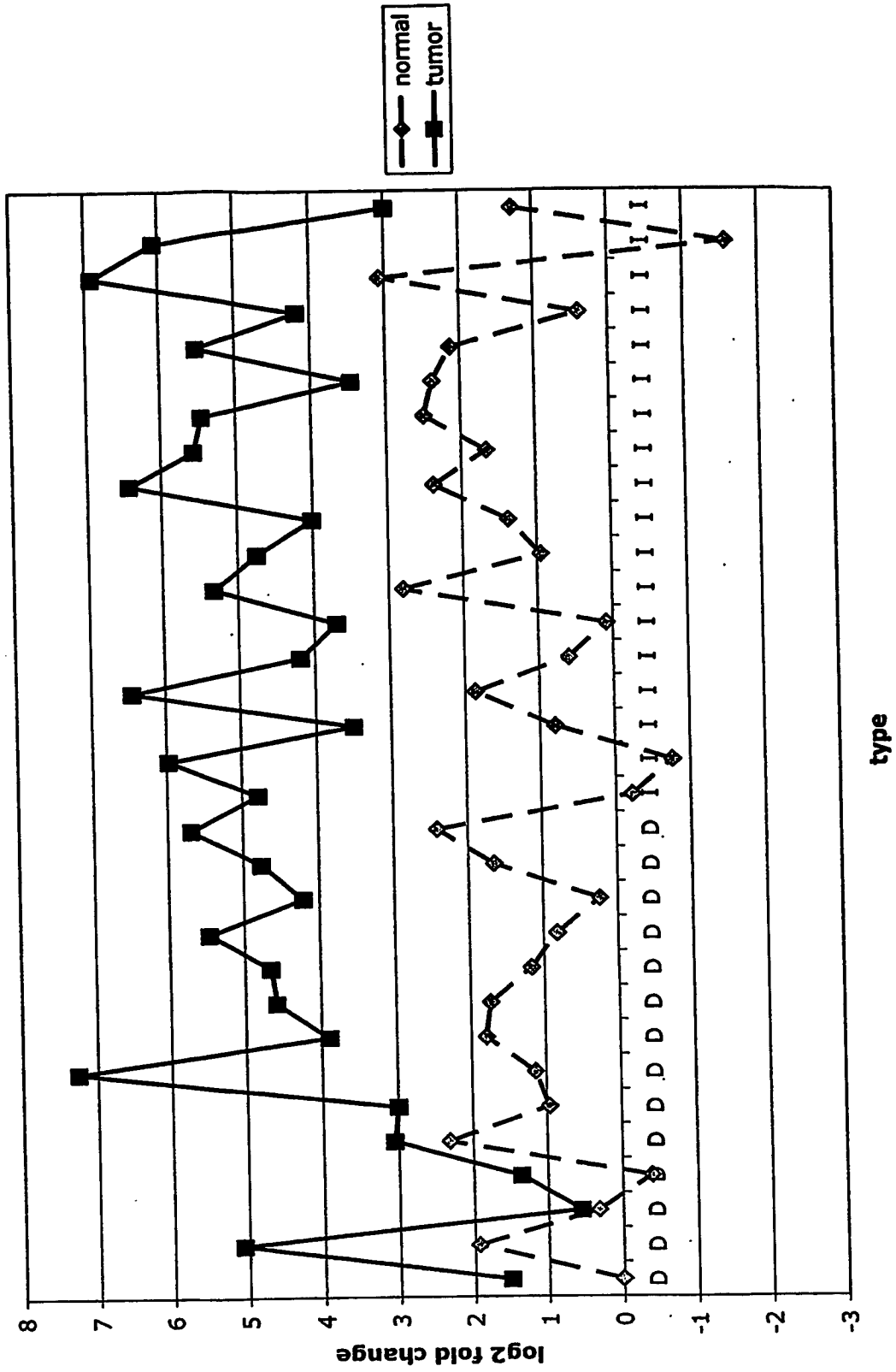


Fig. 11c CSPG2

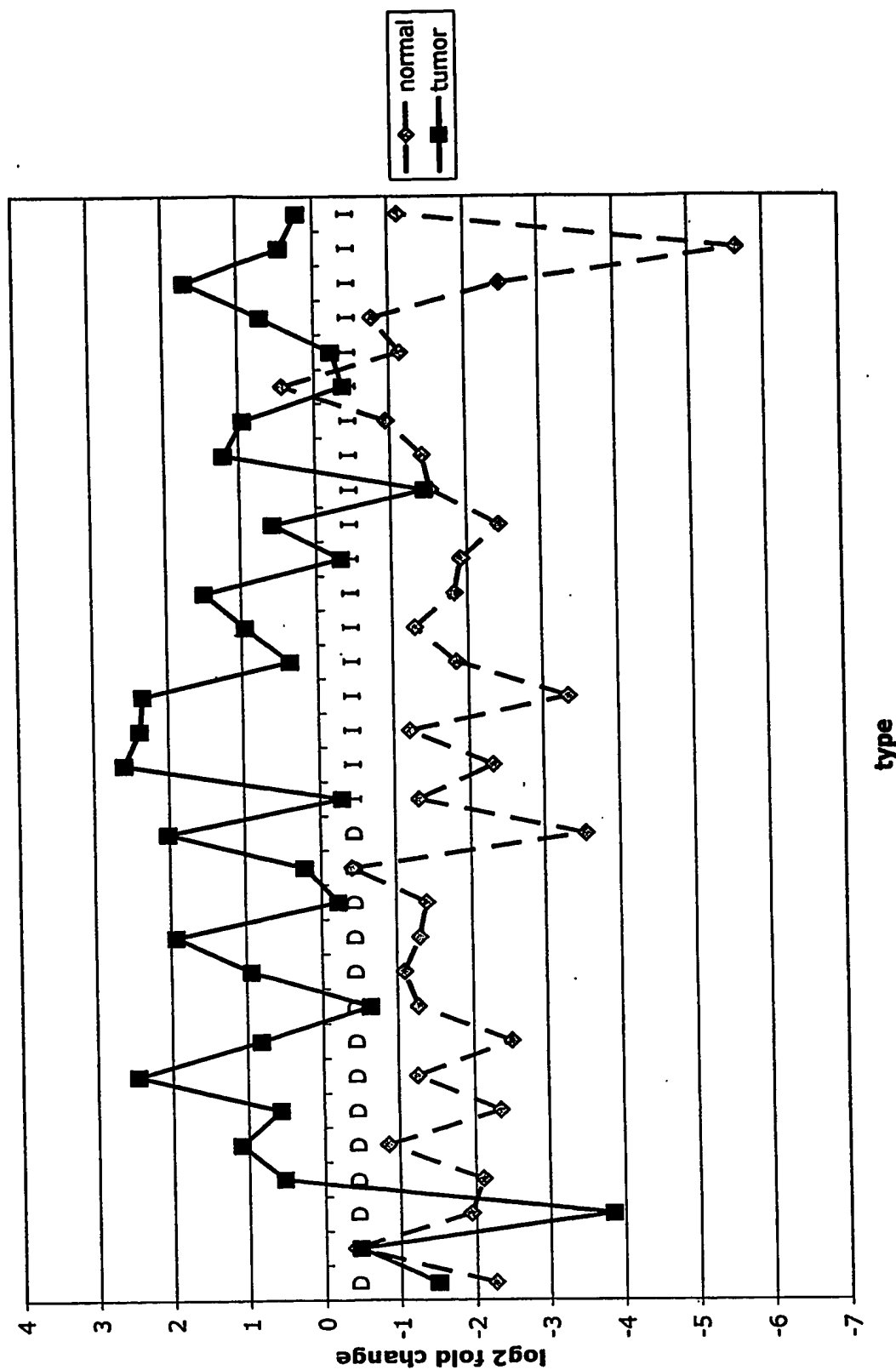


Fig. 11d CST1,2,4

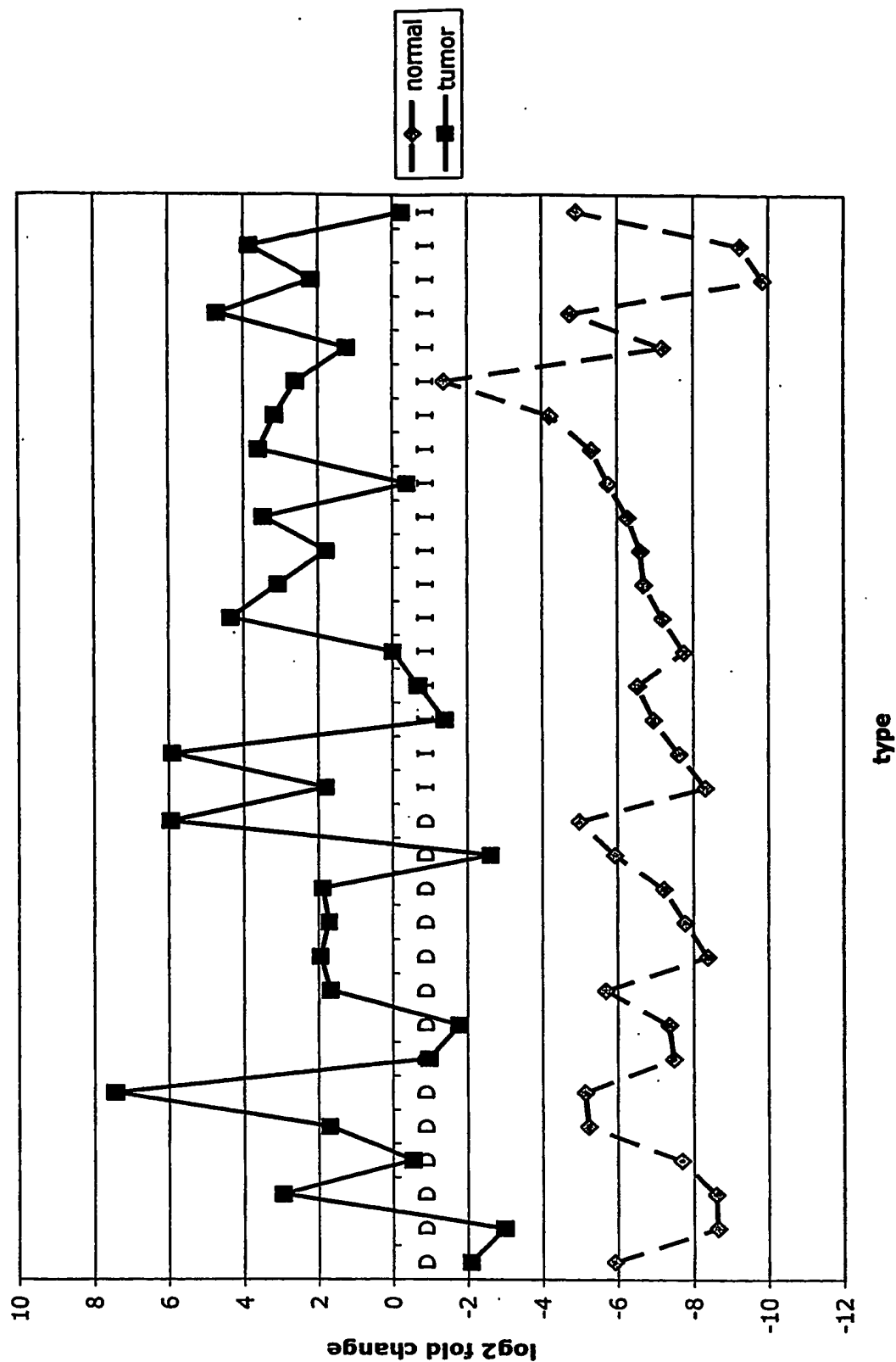


Fig. 11e EFEMP2

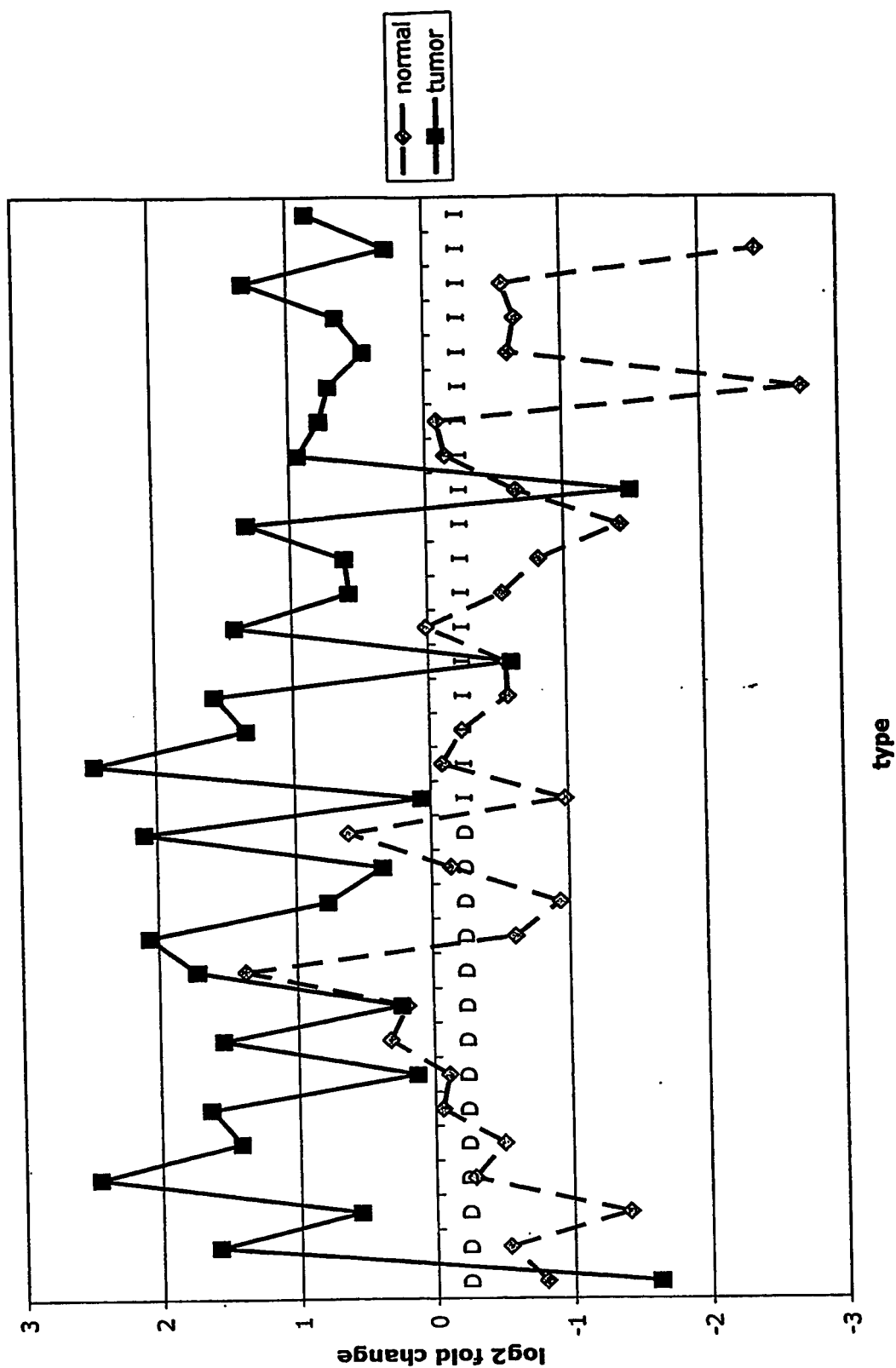


Fig. 11f GGH

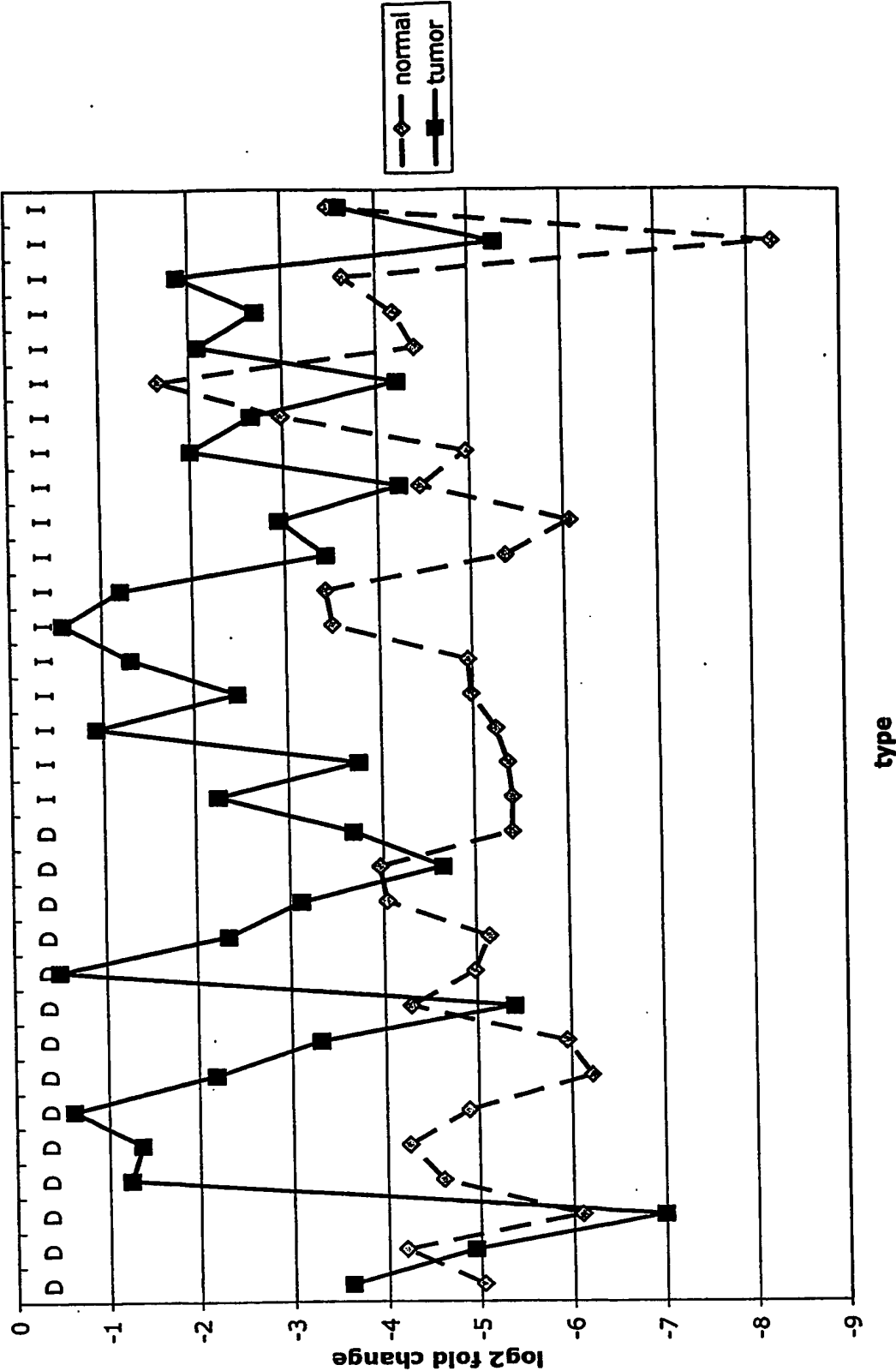


Fig. 11g INHBA

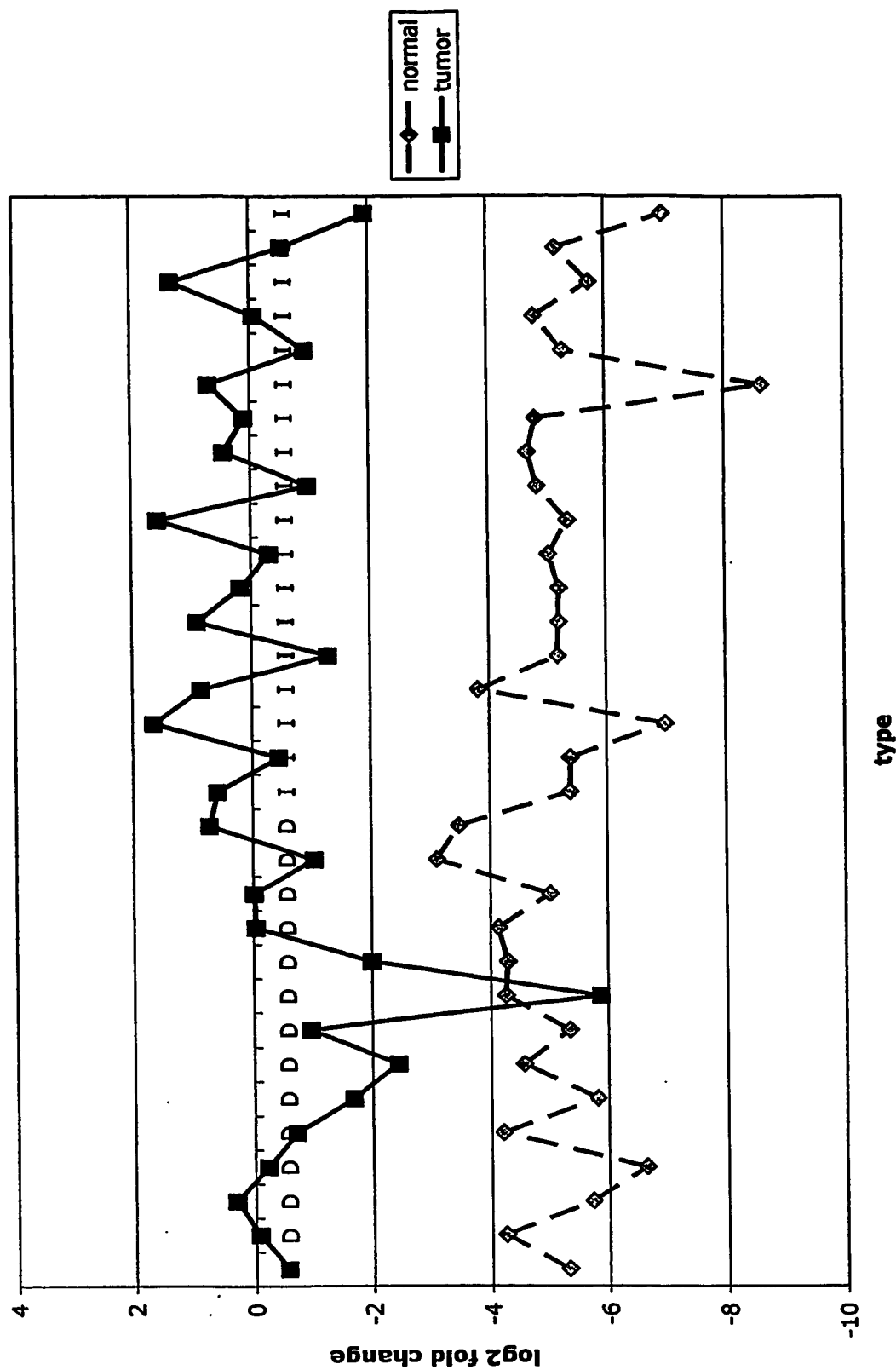


Fig. 11h IGFBP7

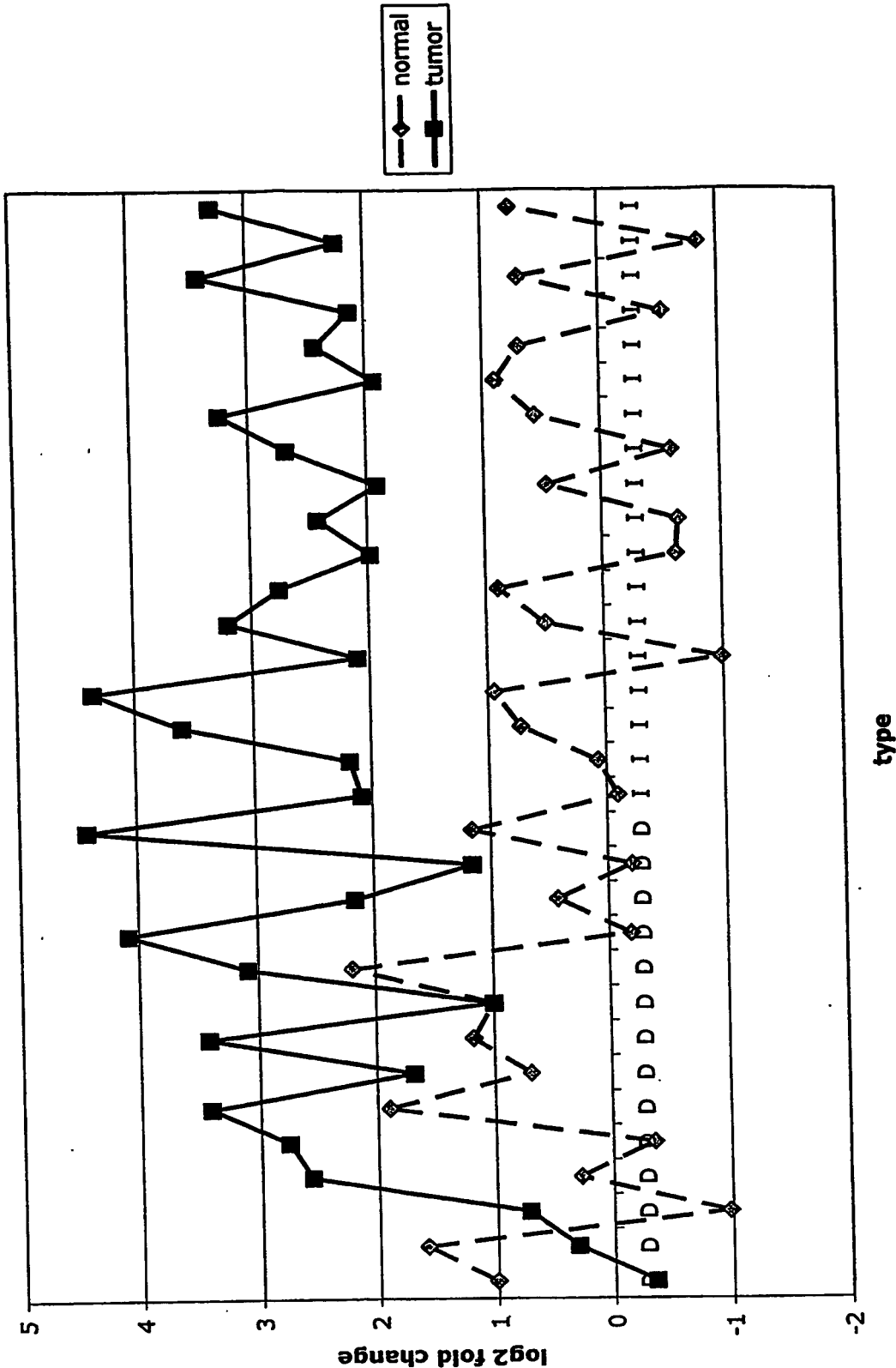


Fig. 11i KLK10

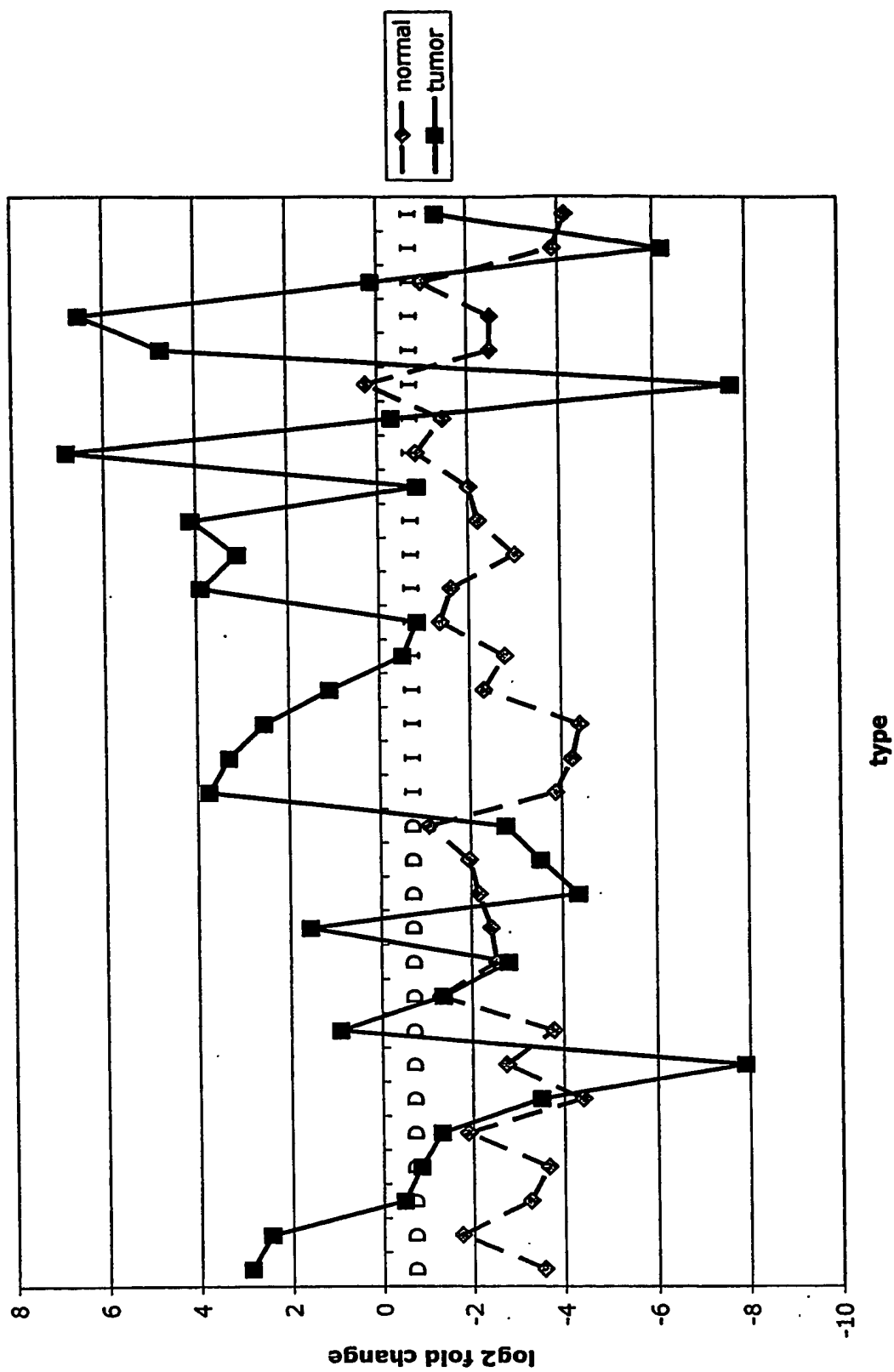
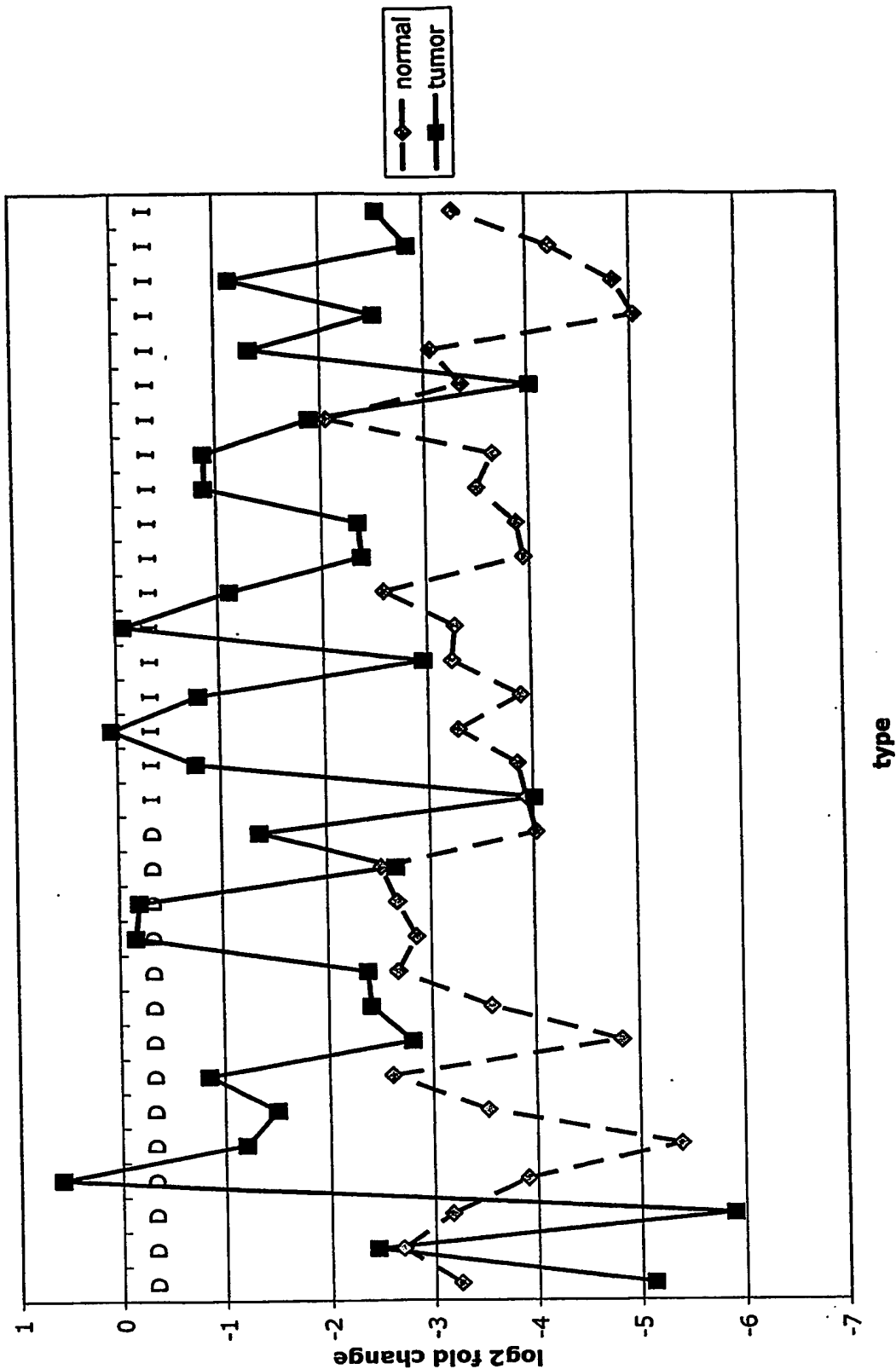


Fig. 11j LEPRE1



80/104

Fig. 11k LUM

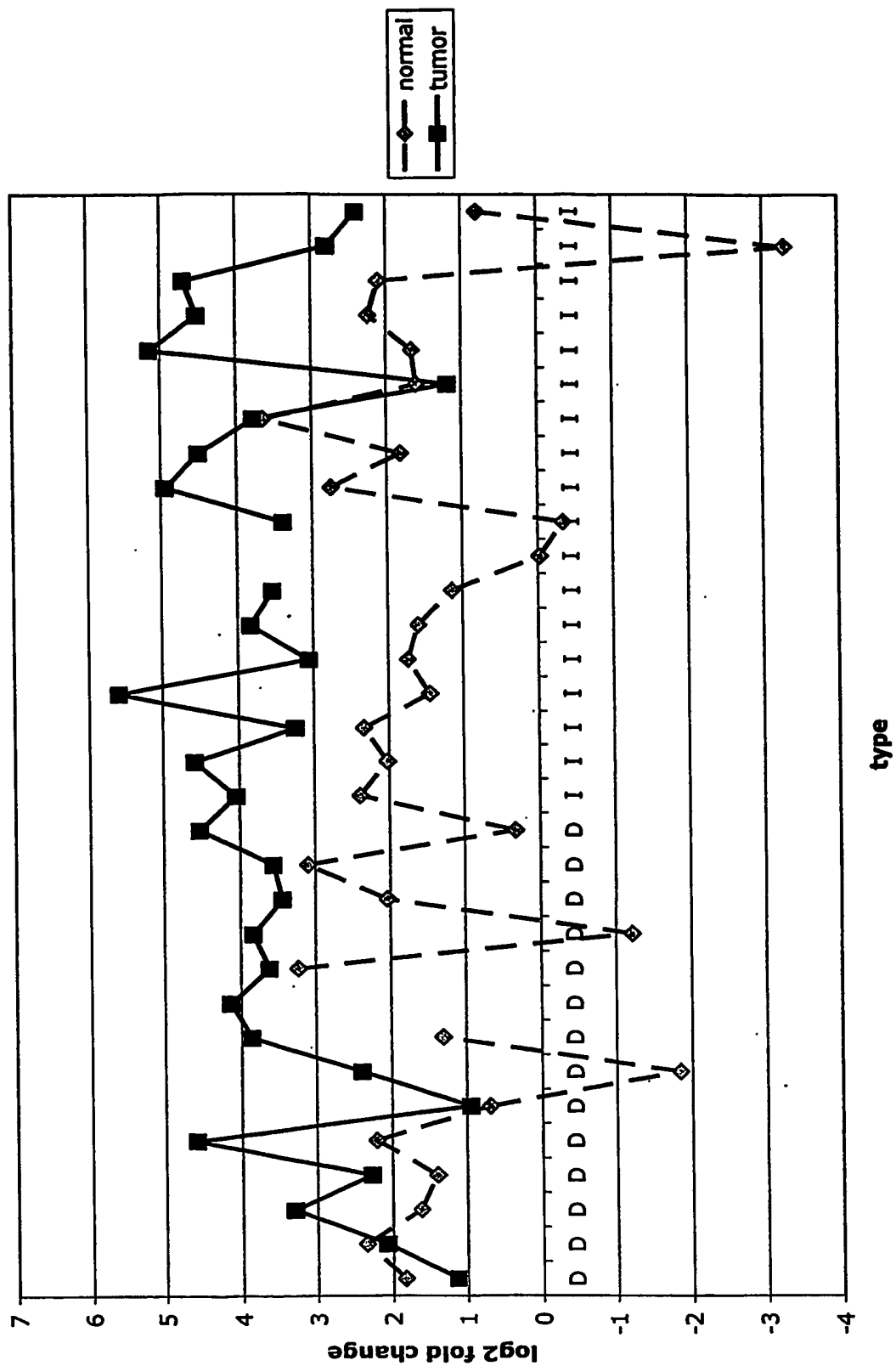


Fig. 11I LOXL2

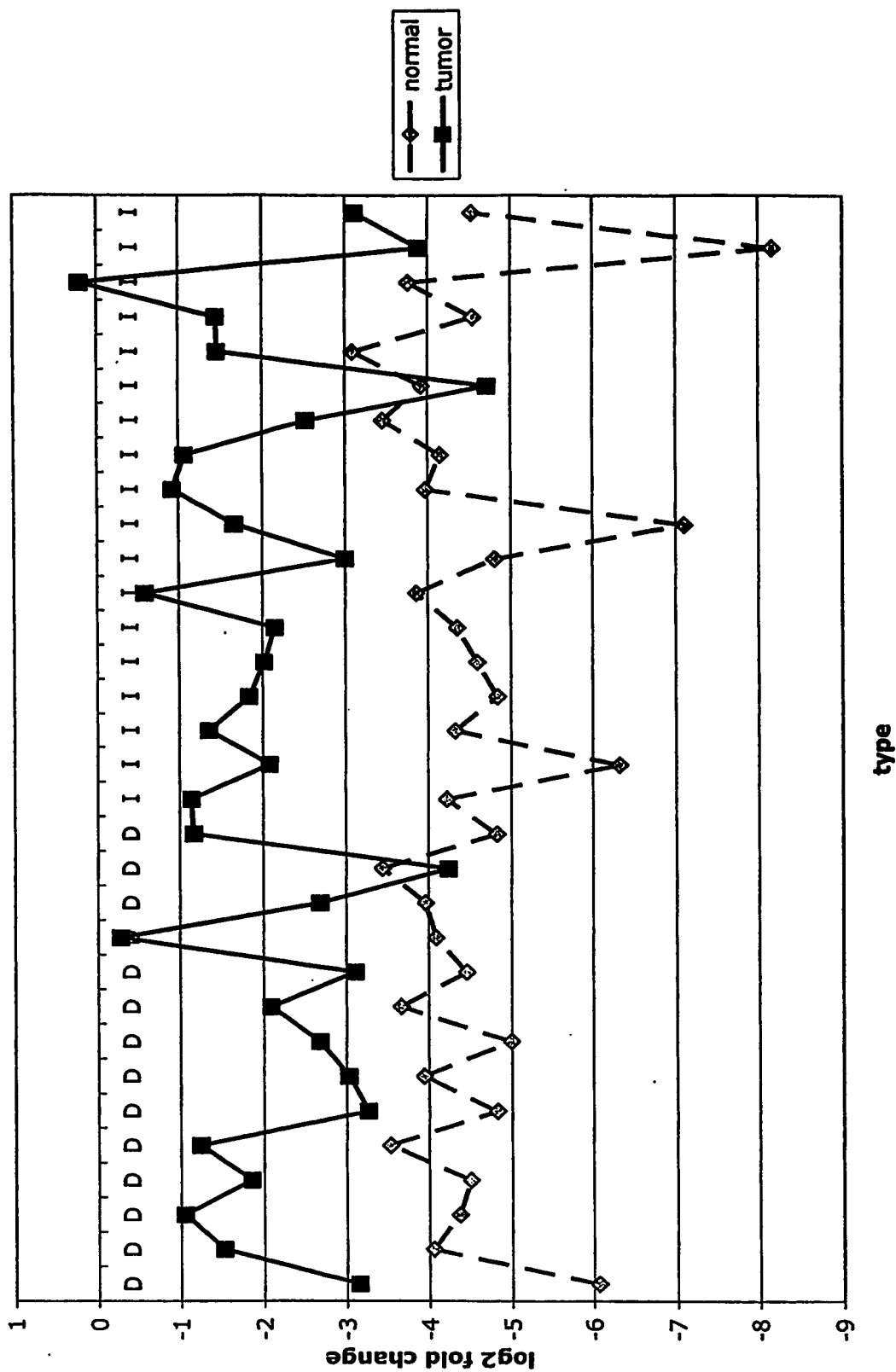


Fig. 11m MMP12

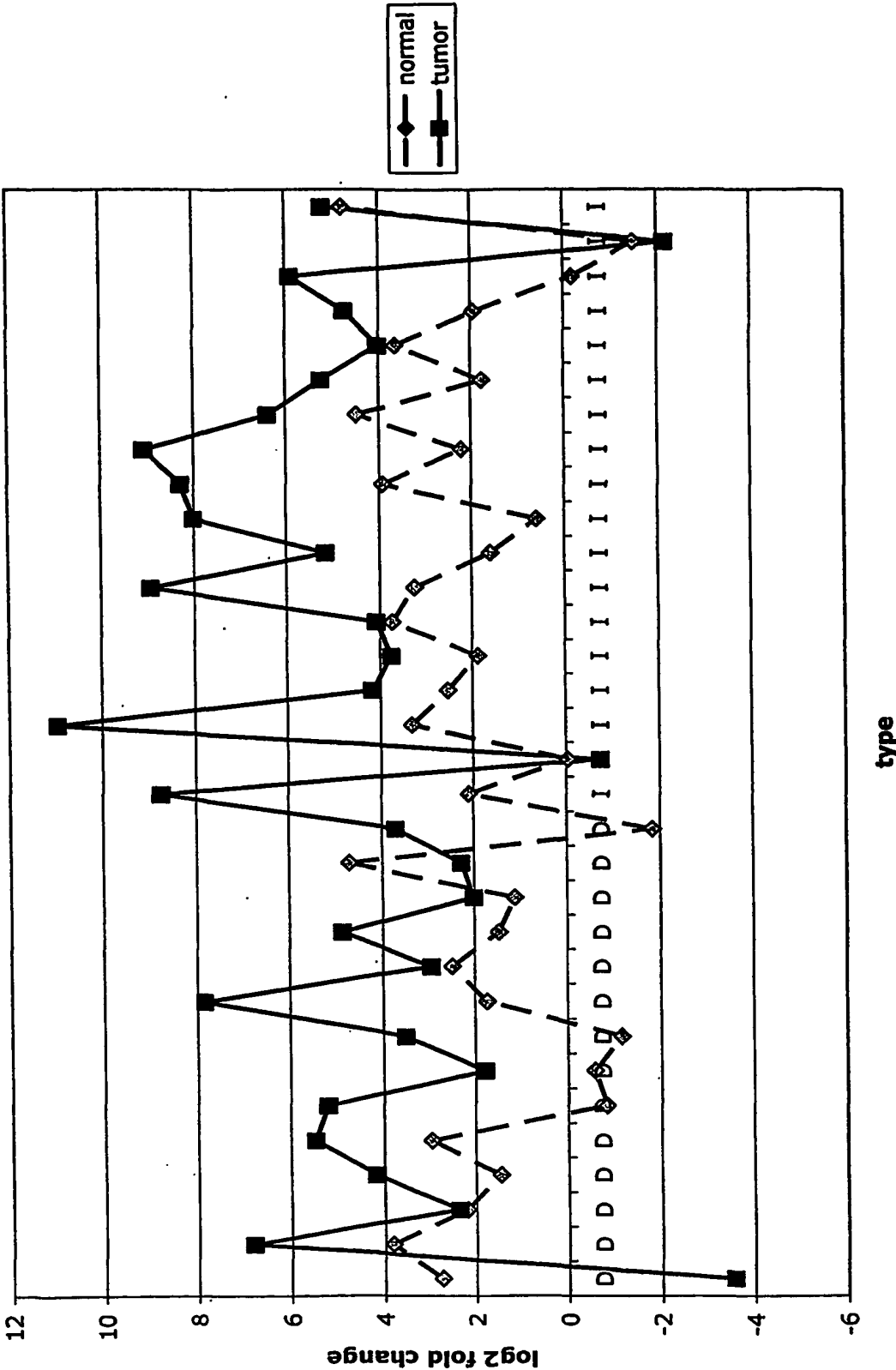


Fig. 11n TIMP1

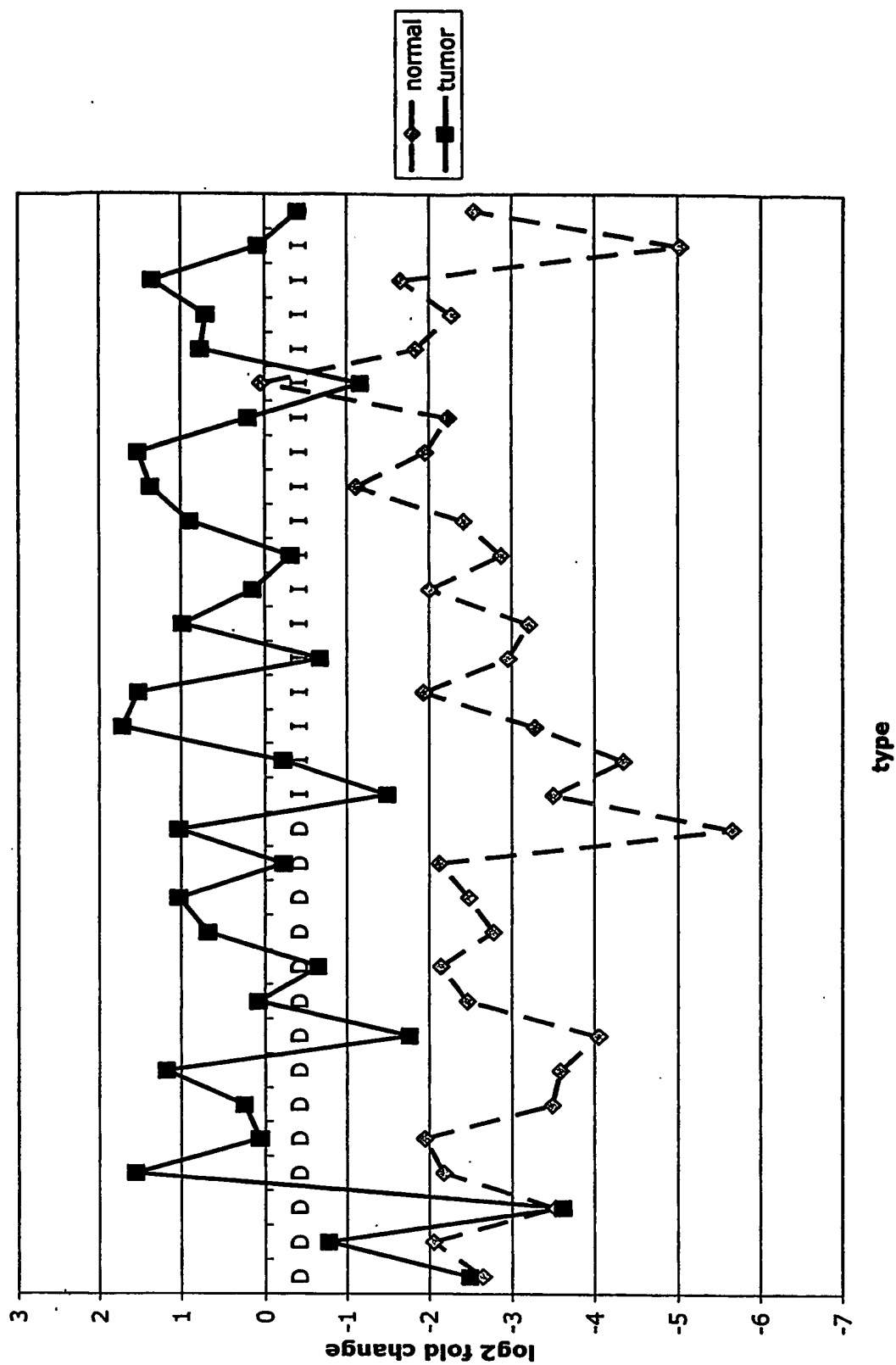


Fig. 11o ASAH1

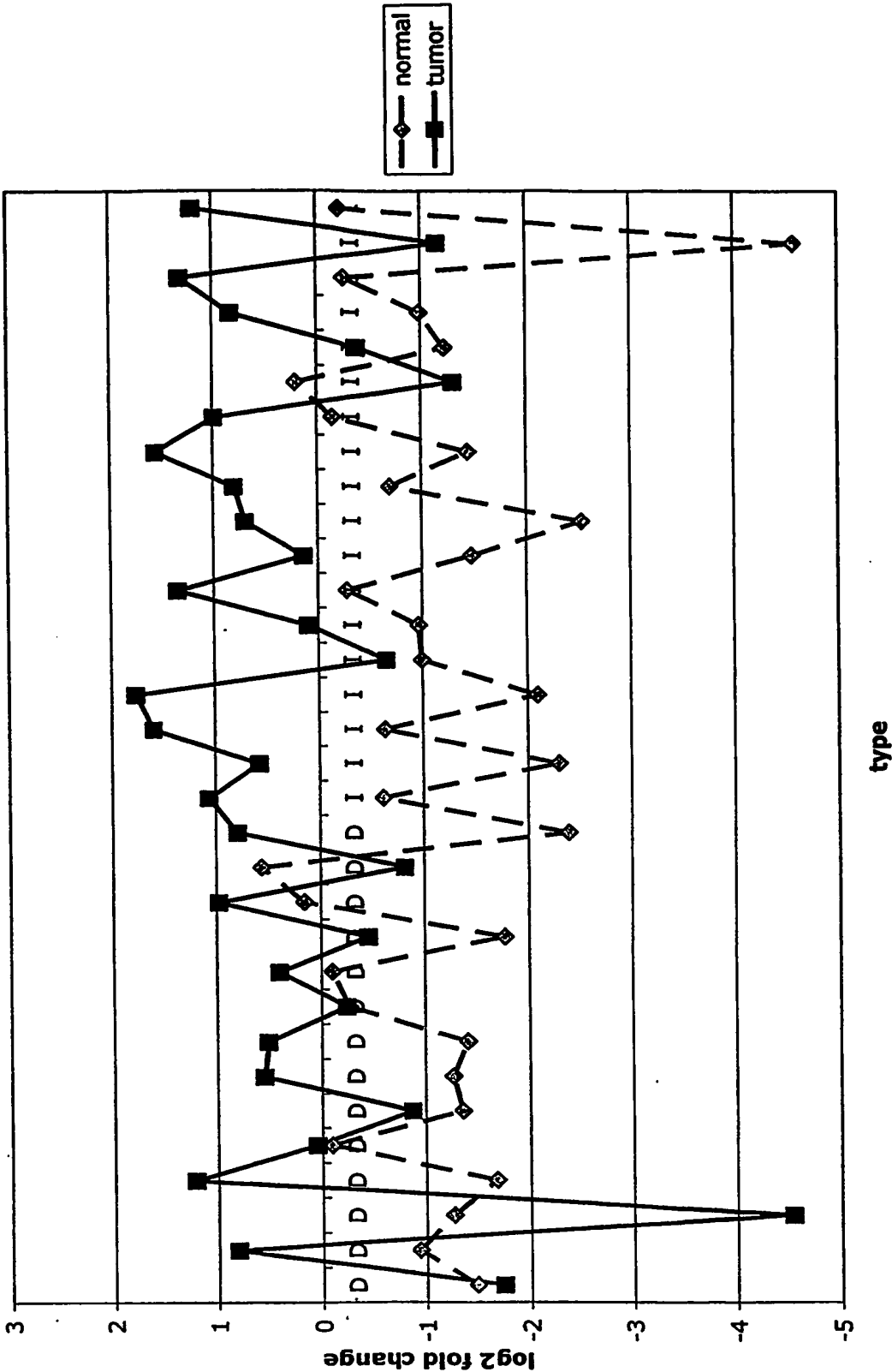


Fig. 11p SPP1

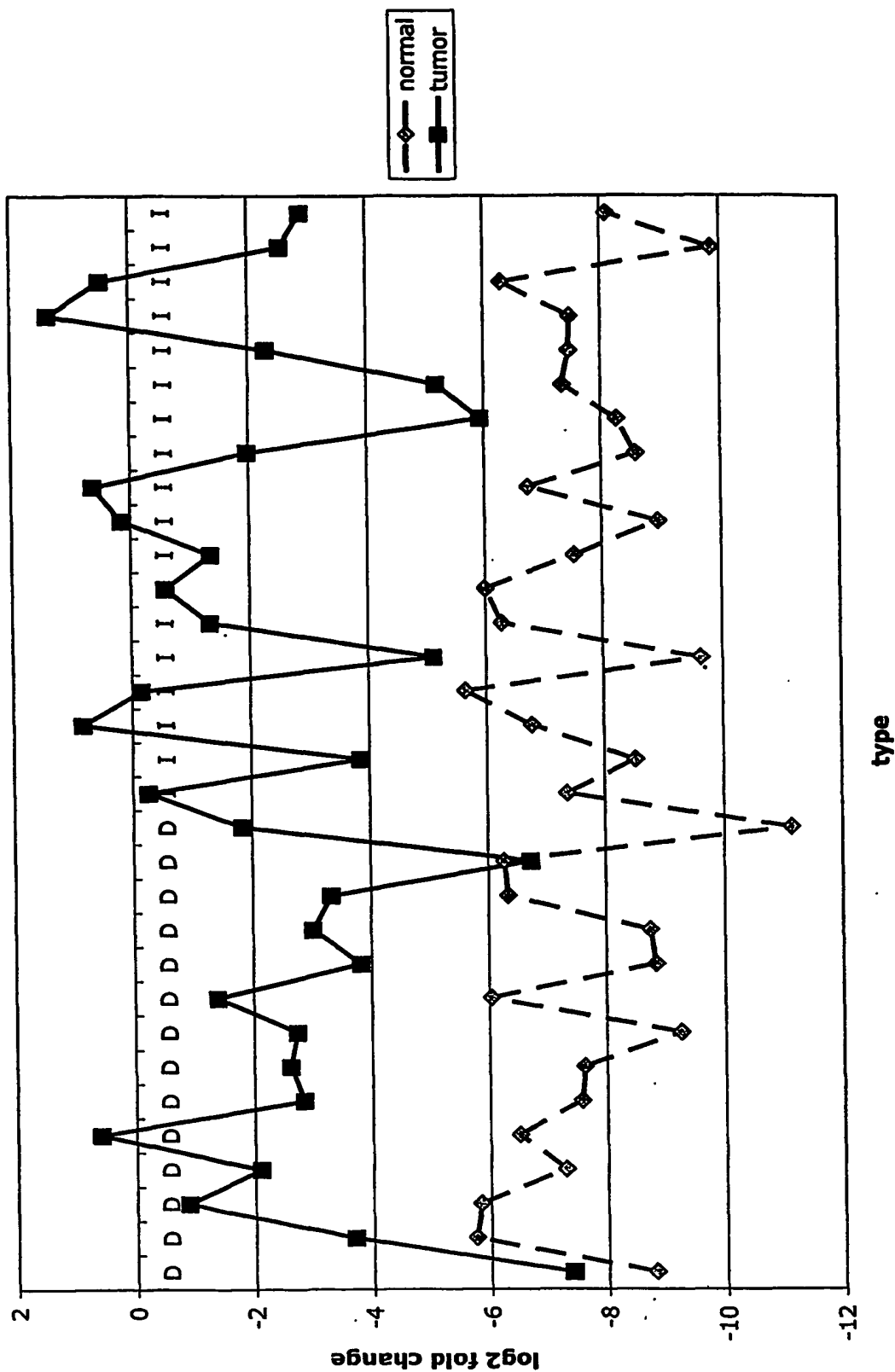


Fig. 11q SFRP2

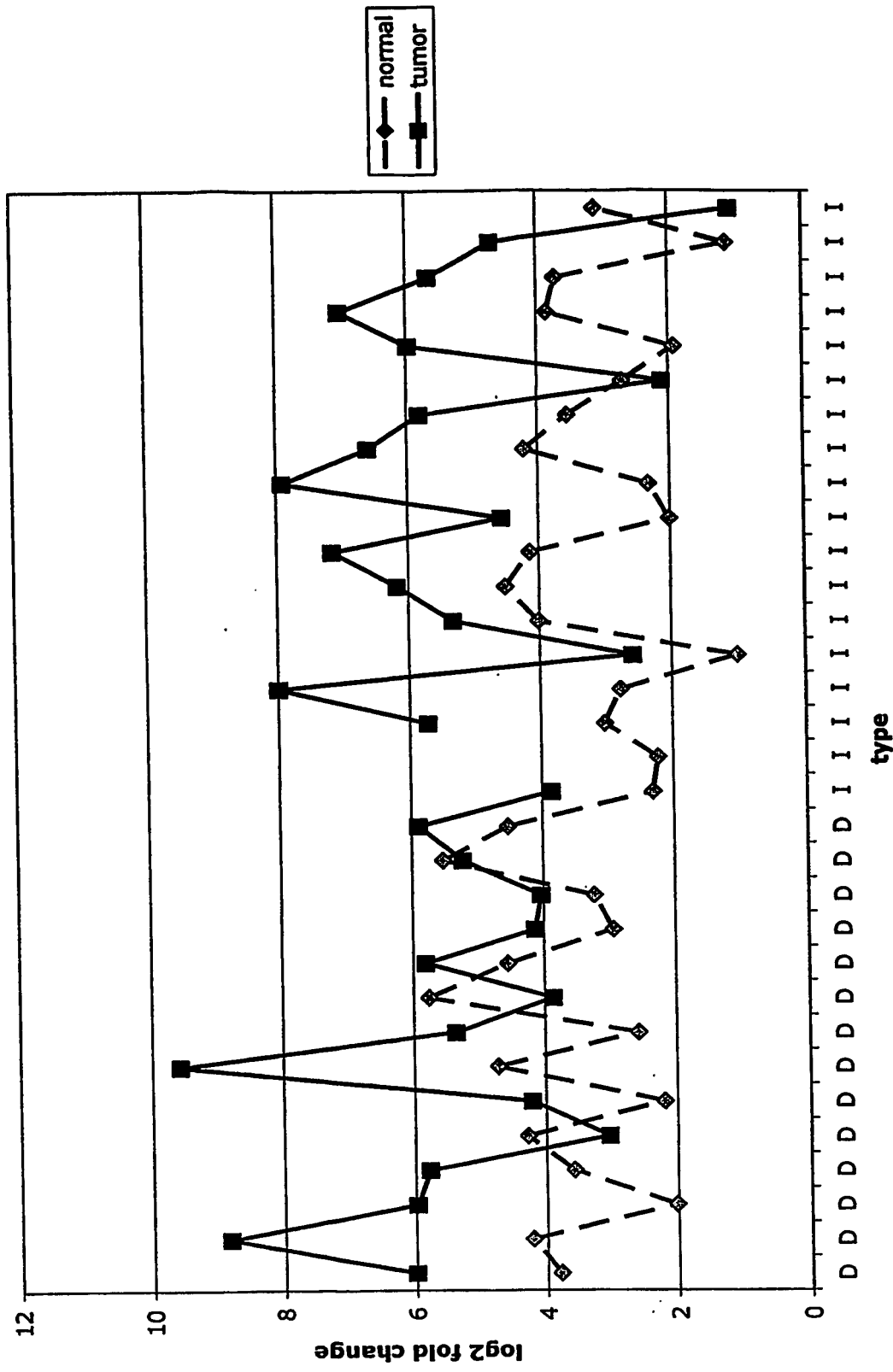


Fig. 11r SFRP4

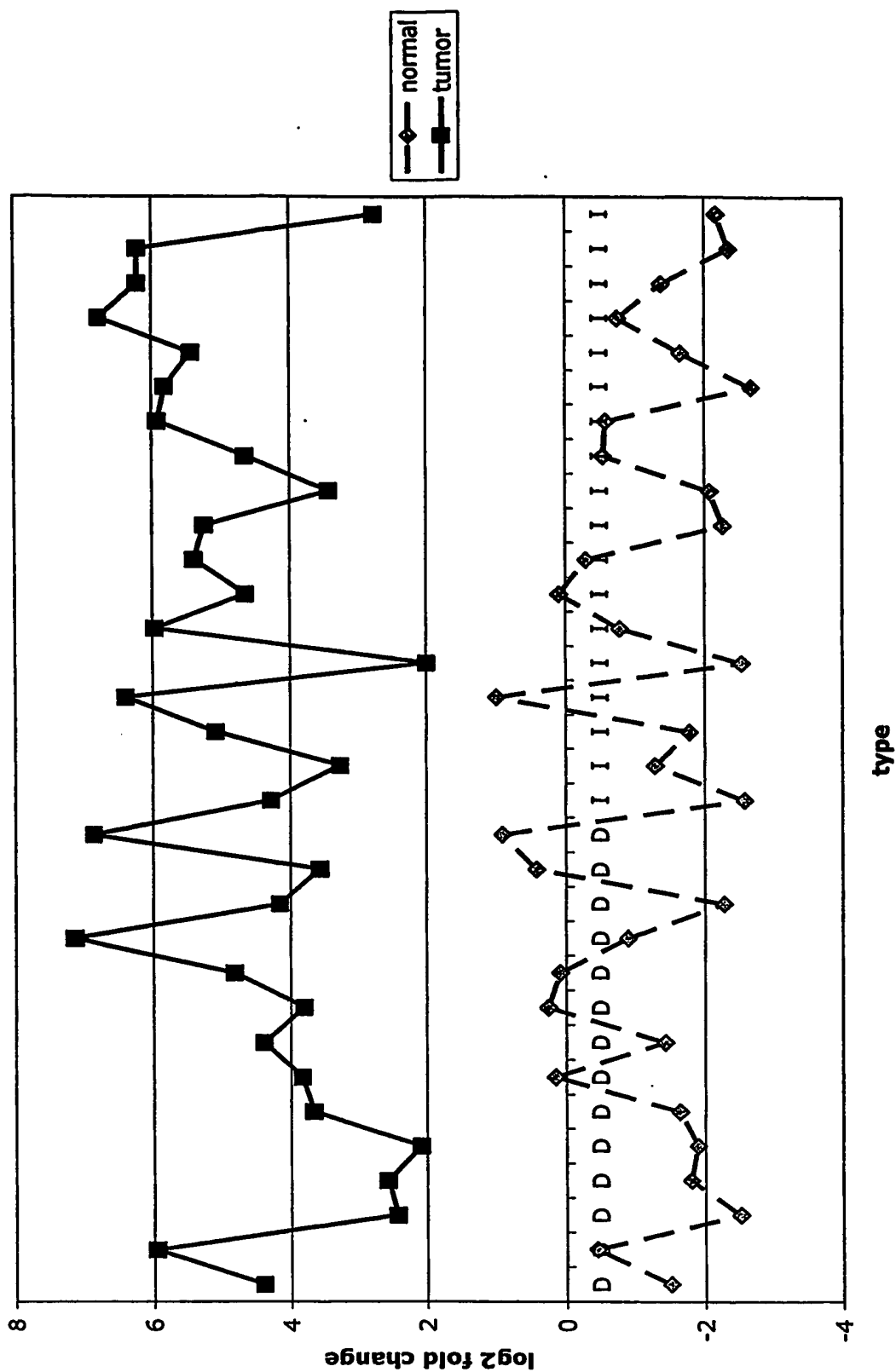


Fig. 11s SPARC

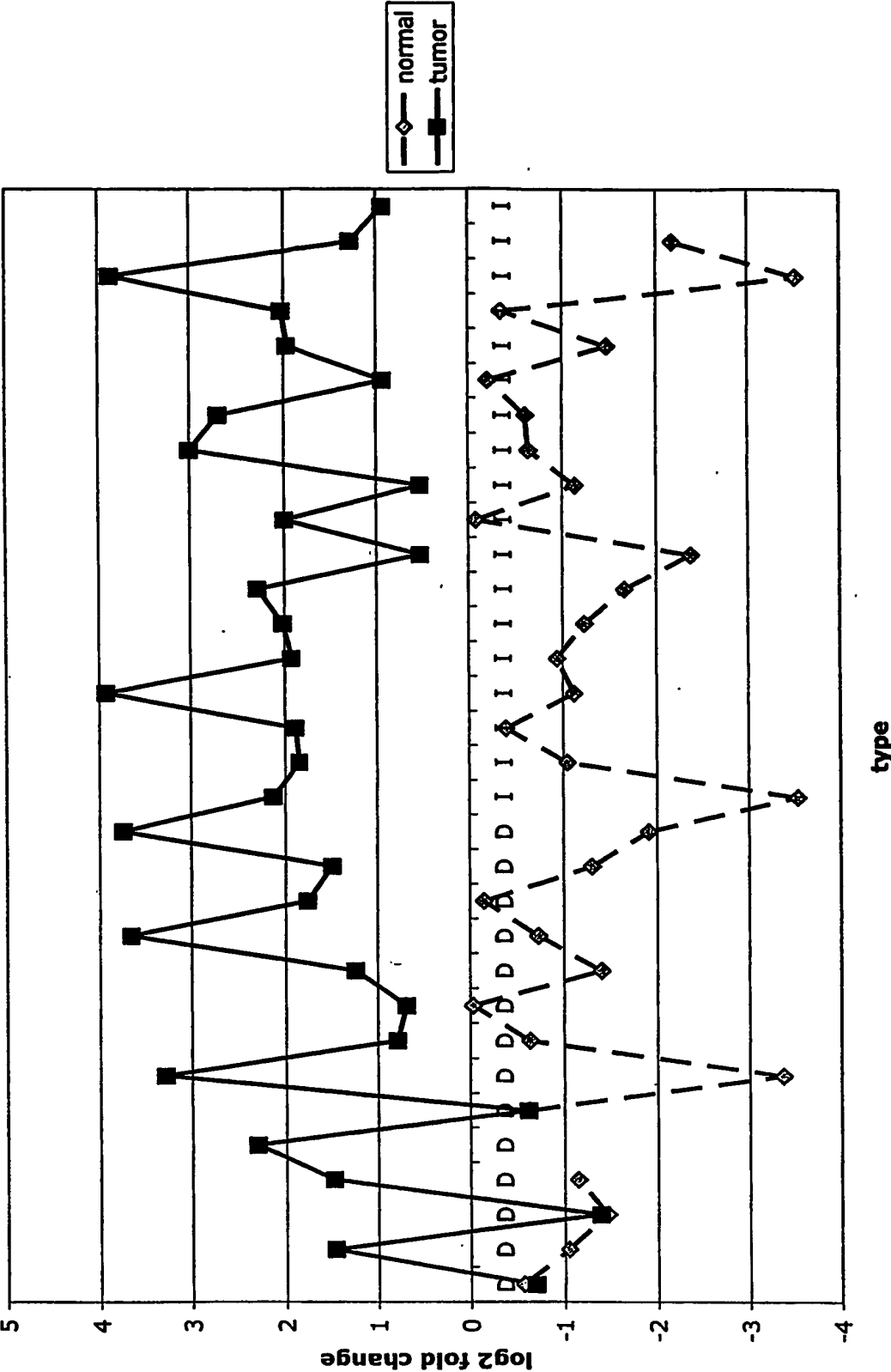


Fig. 11t PRSS11

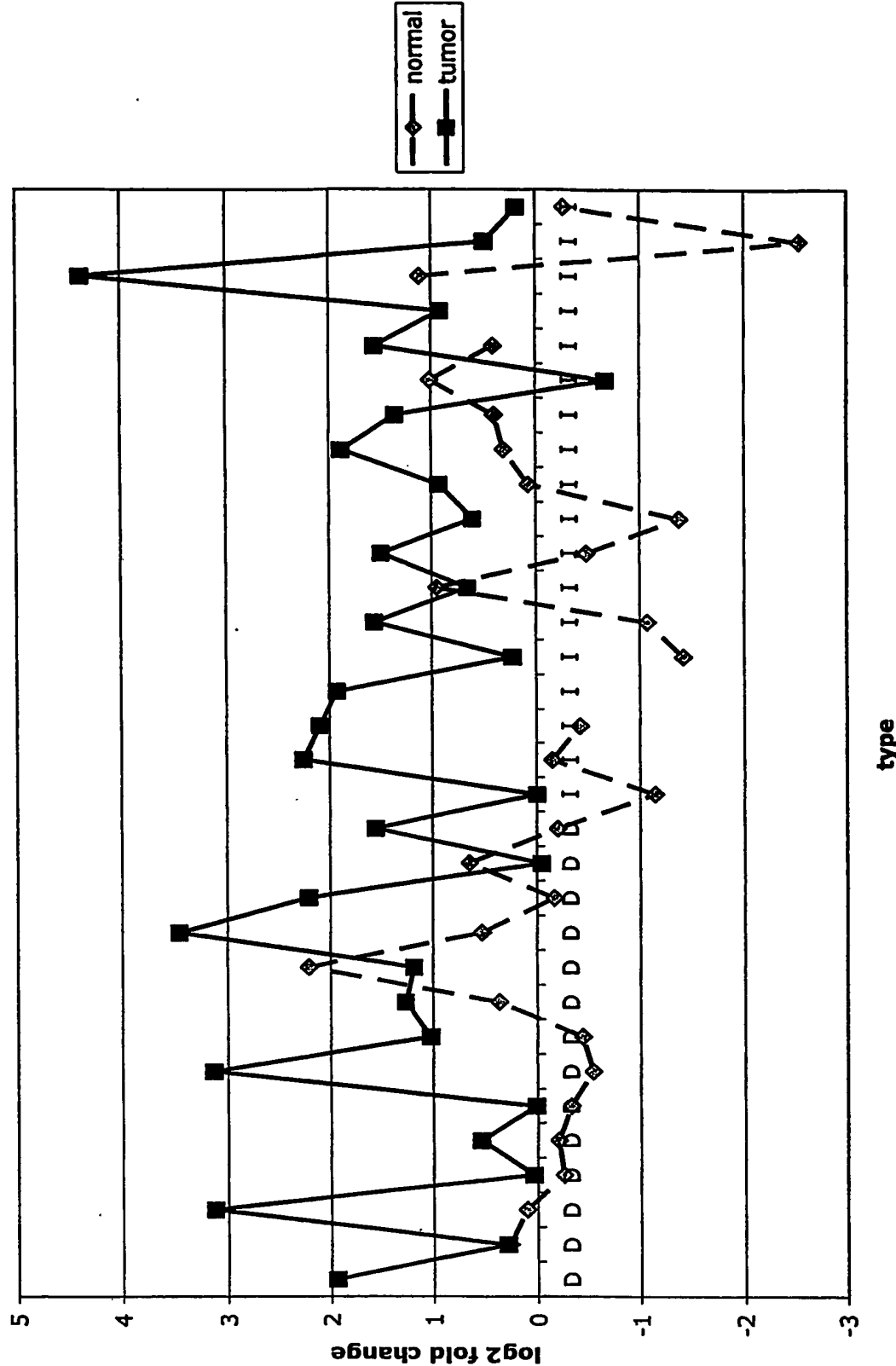


Fig. 11u THBS2

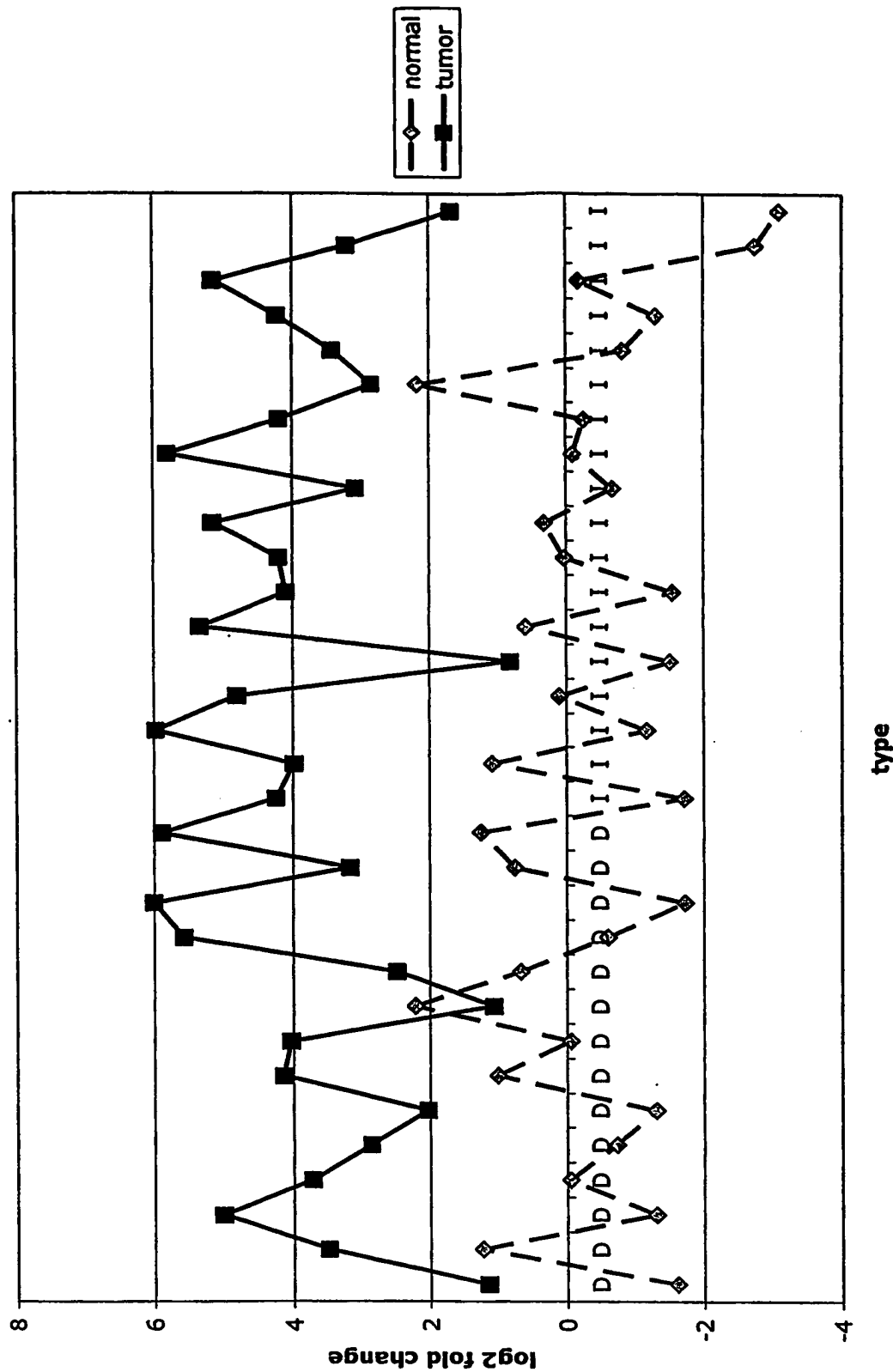


Fig. 11v TG

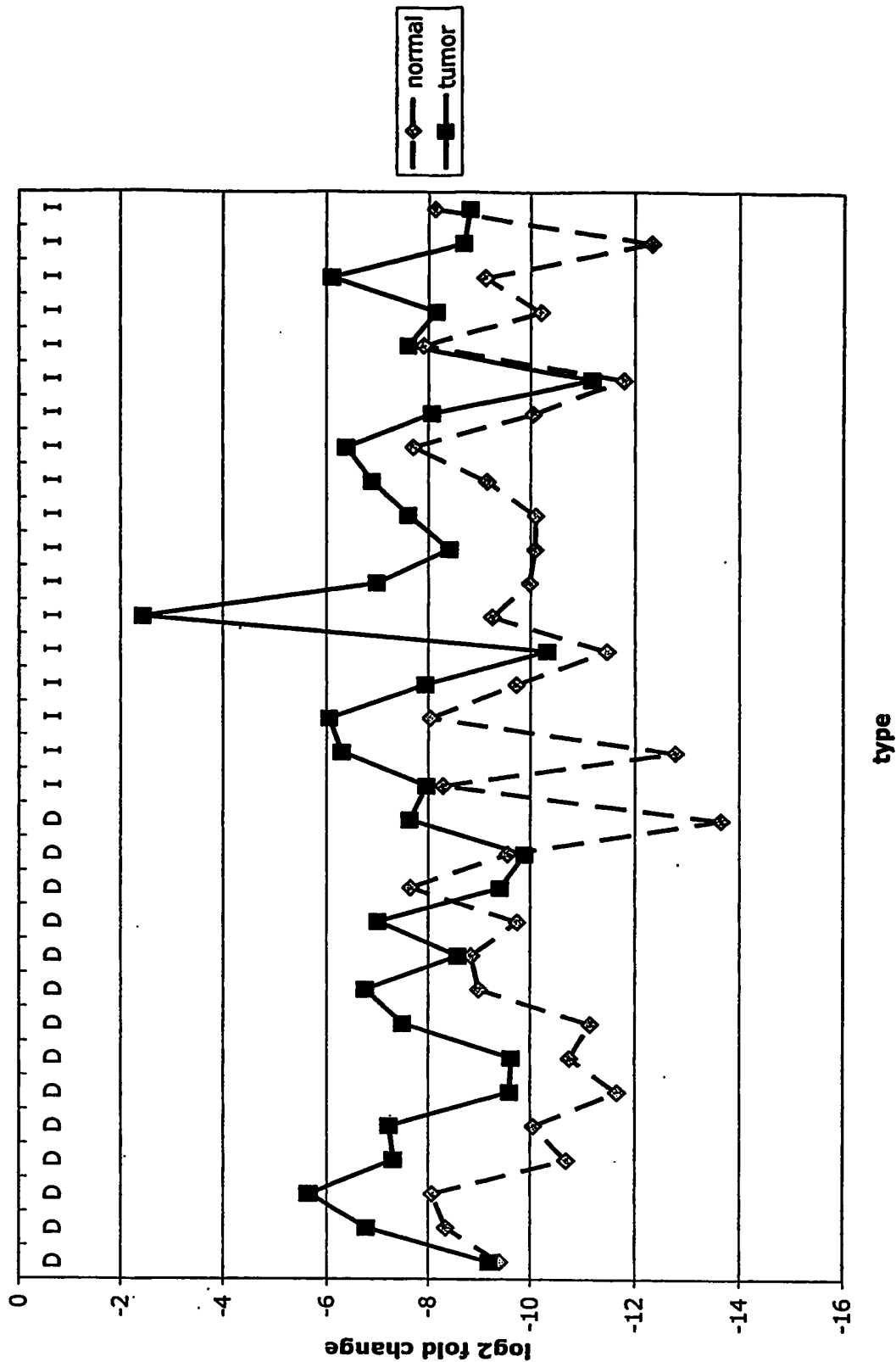


Fig. 11w TGFBI

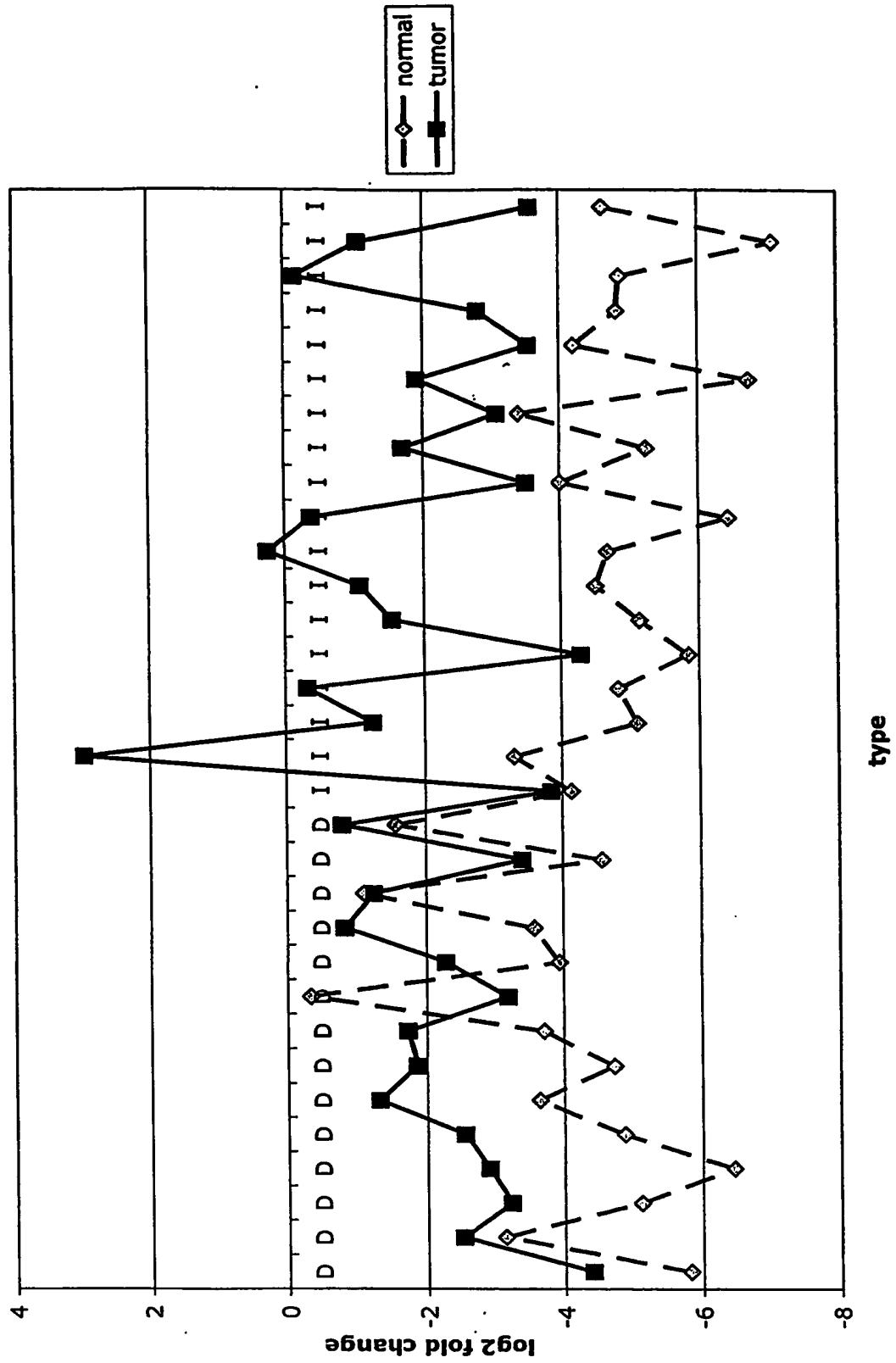


Fig. 11x CGR11

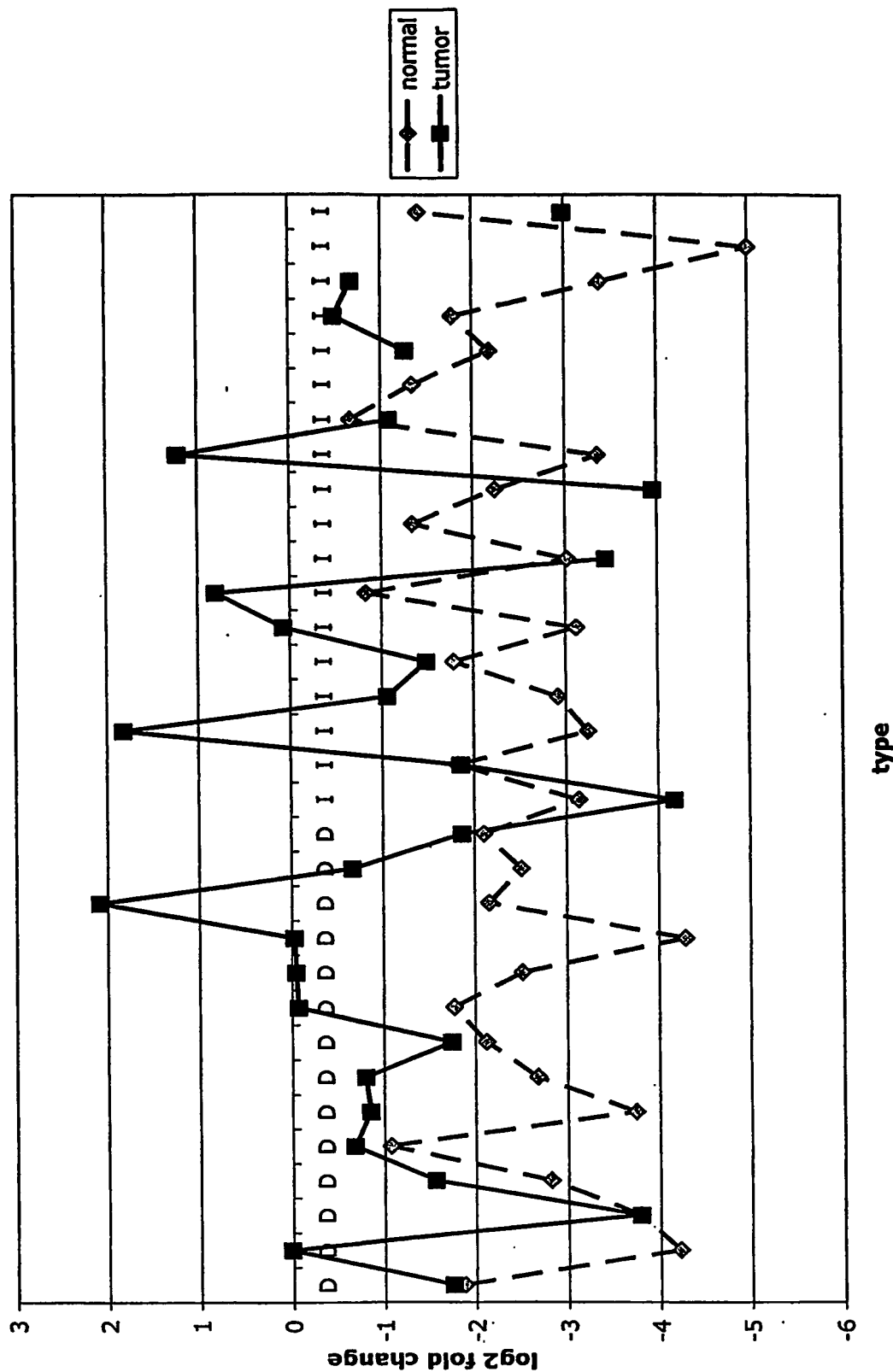
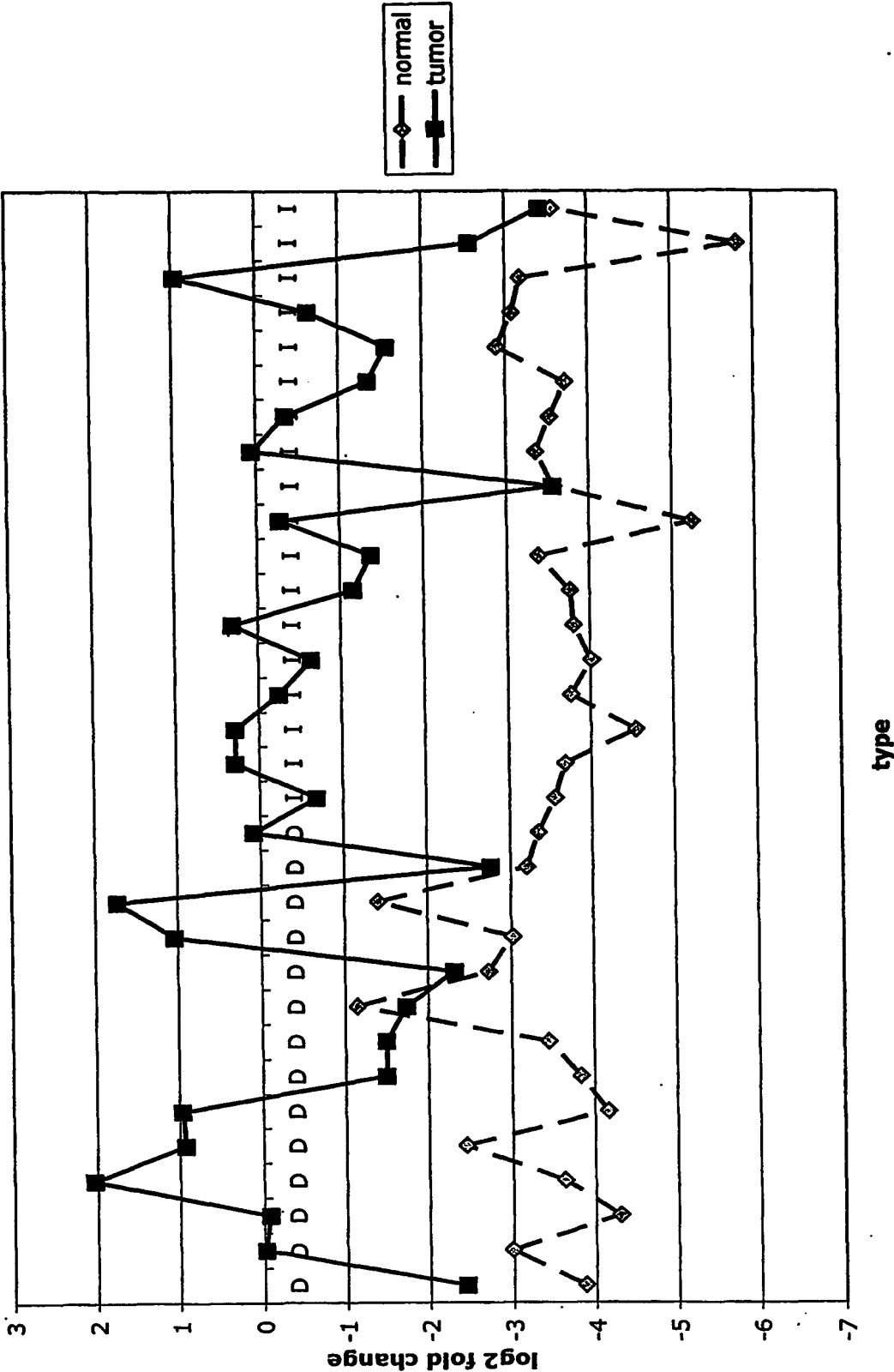


Fig. 11y SERPINH1



95/104

Fig. 11z MMP2

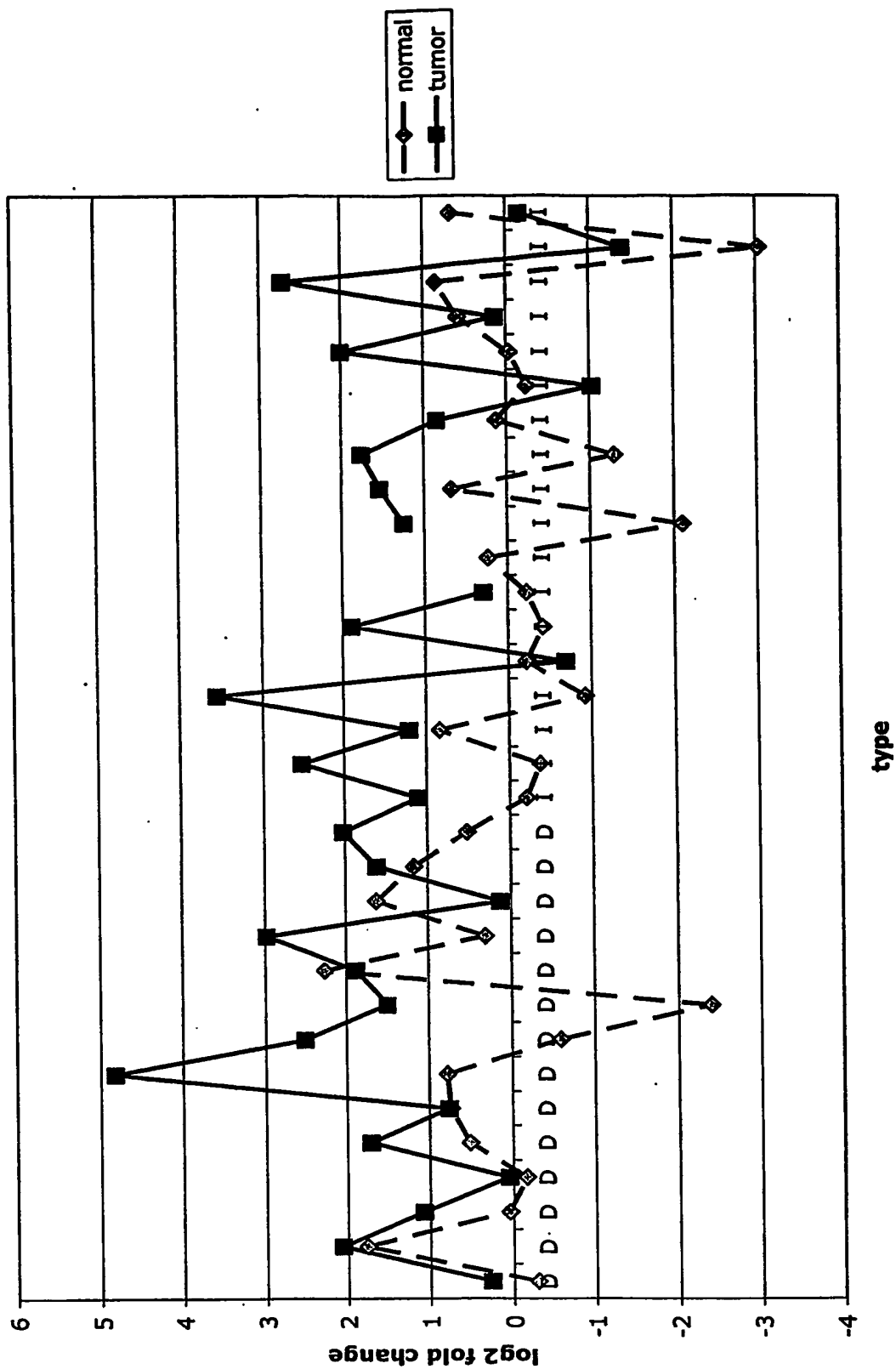


Fig. 11aa PCSK5

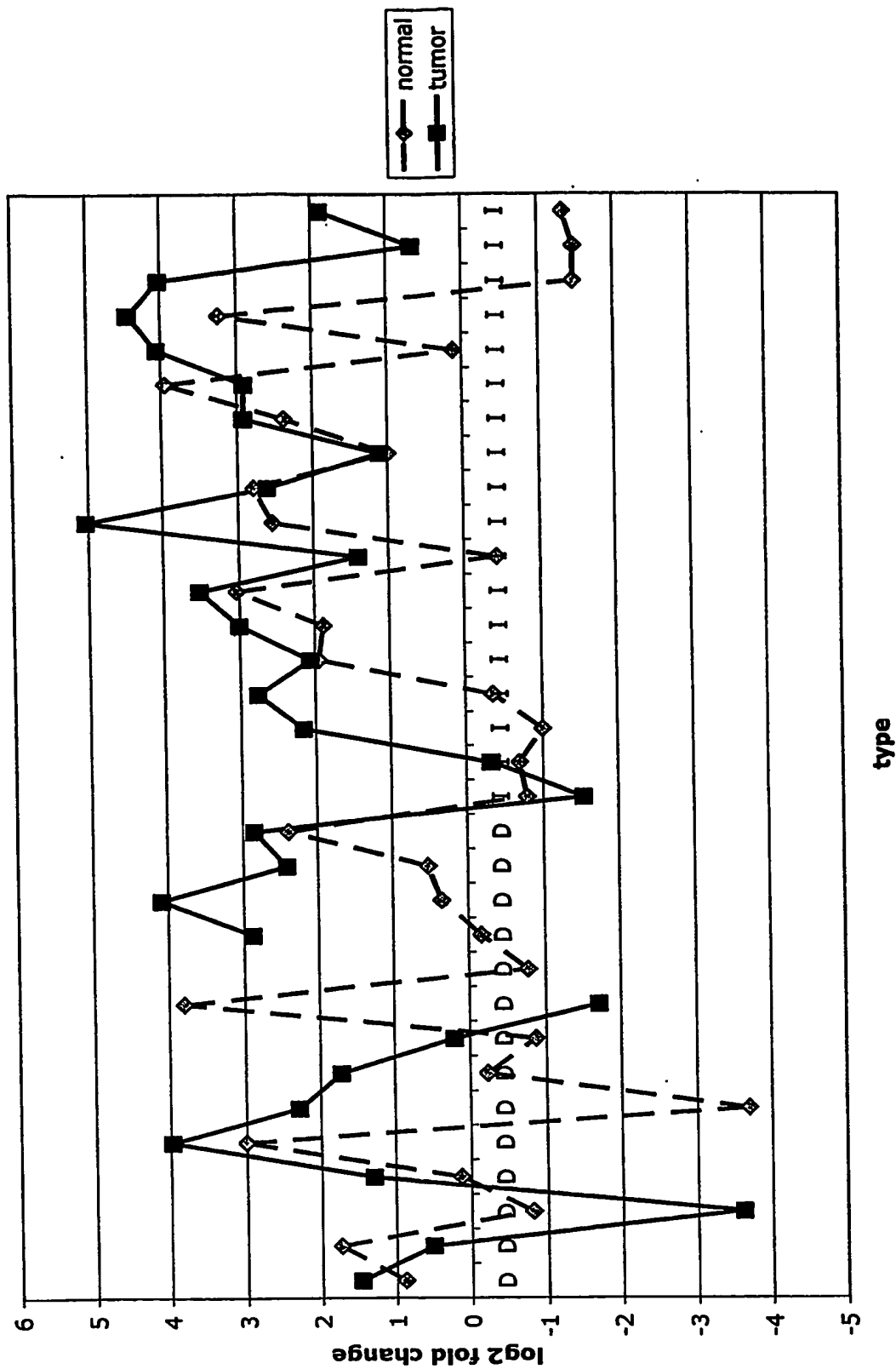


Fig. 11ab SERPINB5

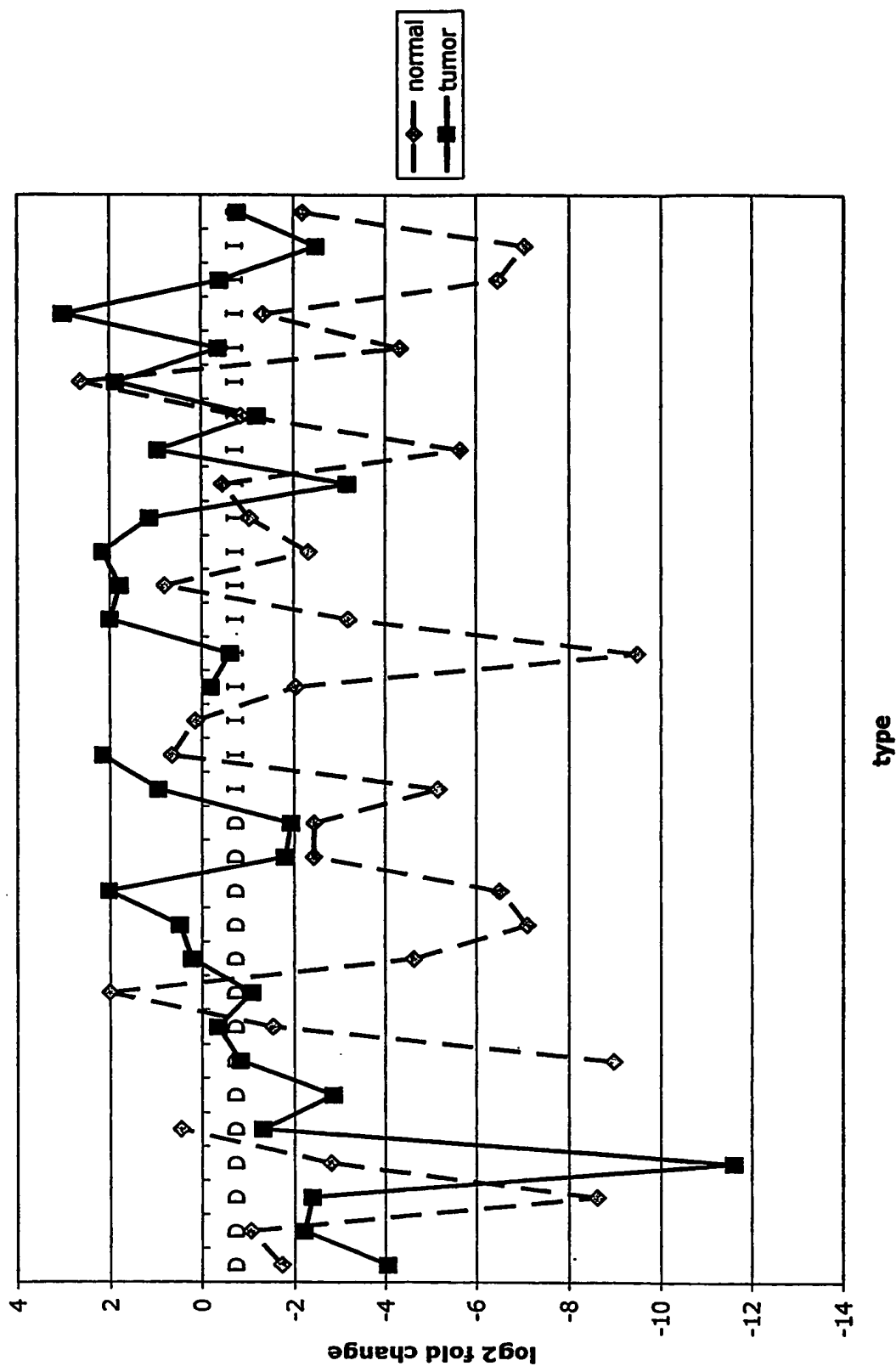


Fig. 11ac TGFB1

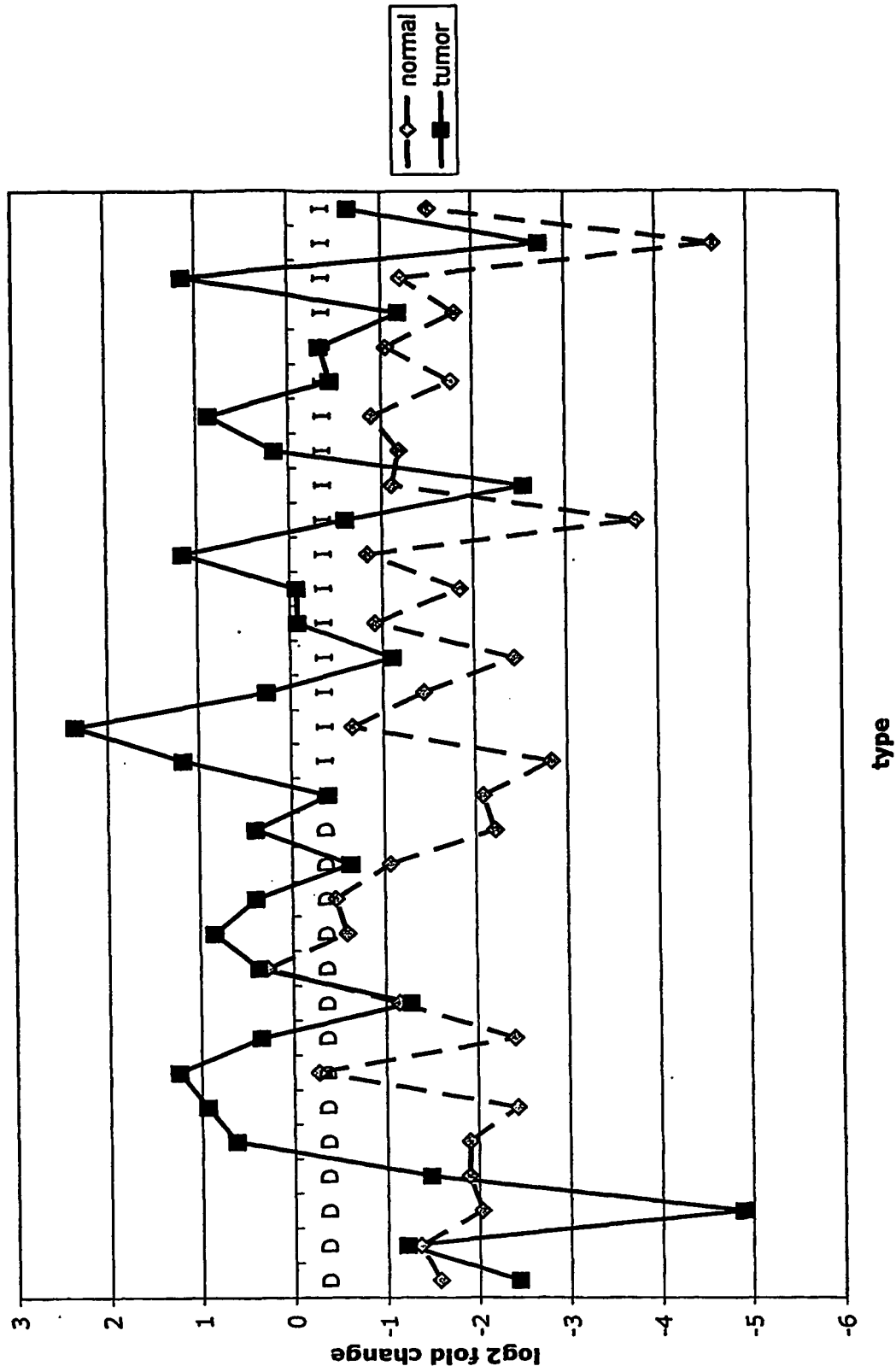


Fig. 11ad CEACAM5

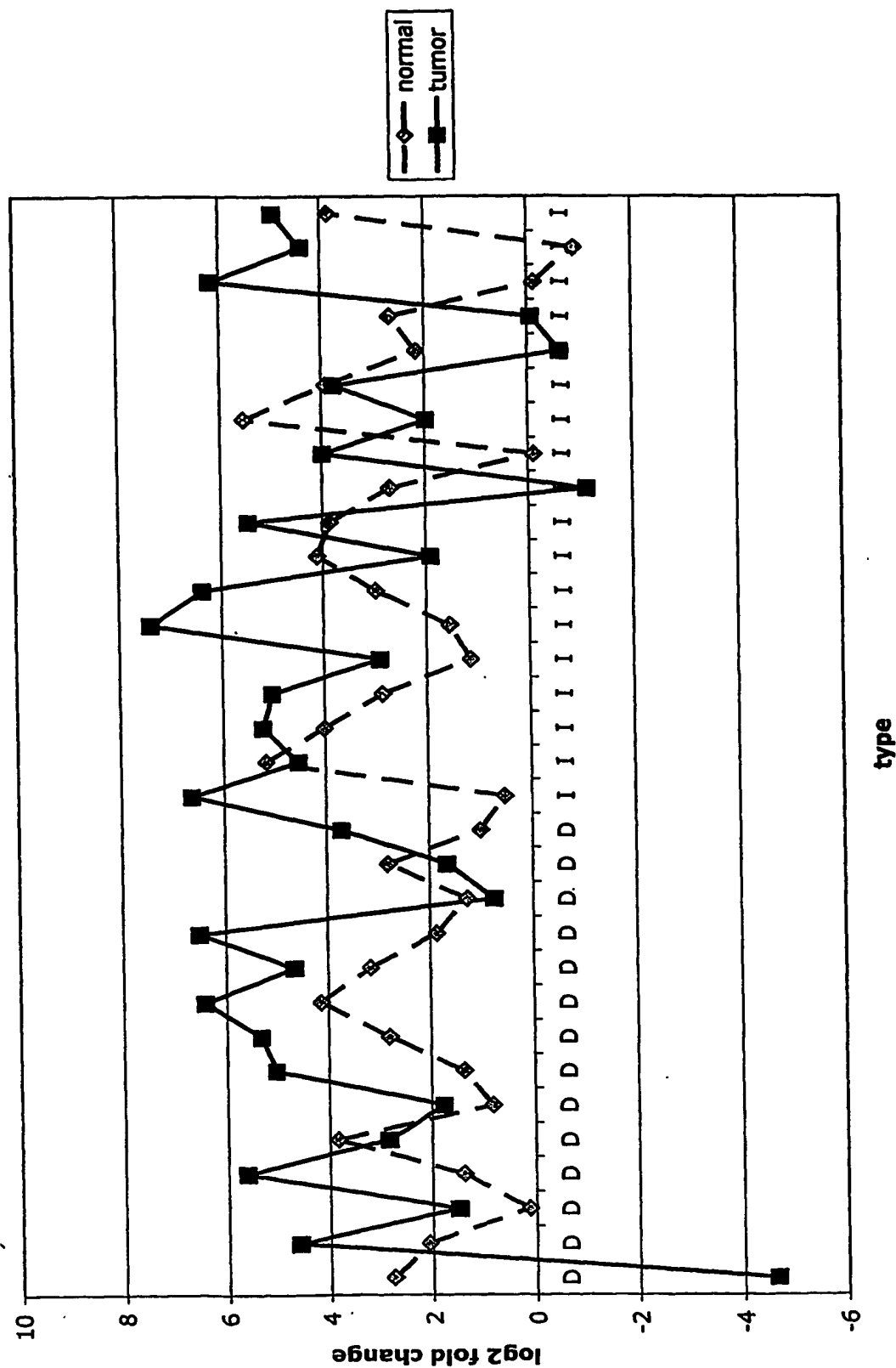
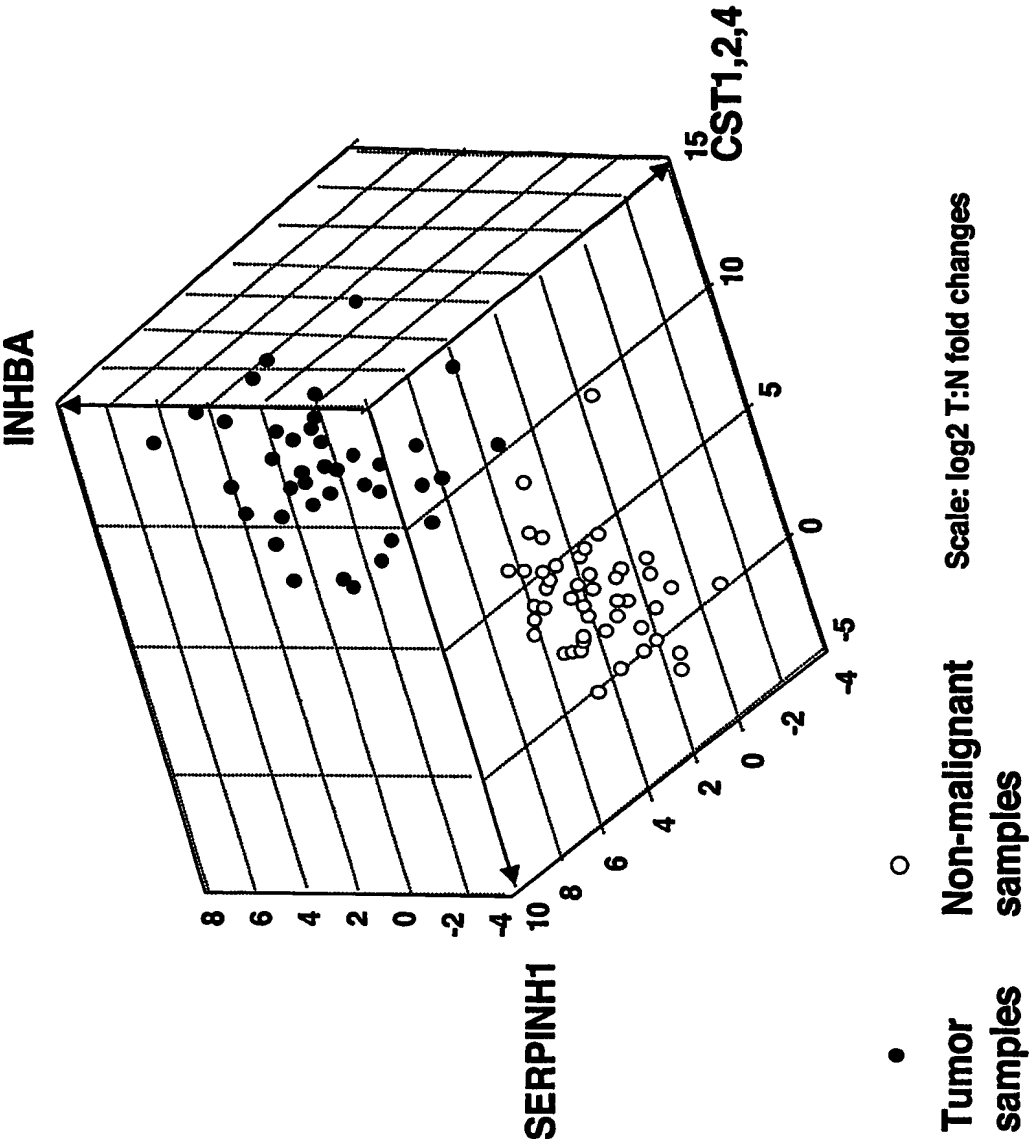


Fig. 12 The separation of gastric tumor samples from non-malignant samples using three markers



Number of markers in test	Total possible tests	Number of tests with sensitivity				Proportion of tests with sensitivity			
		>=90%	>=95%	>=99%		>=90%	>=95%	>=99%	
1	29	2	1	0		6.9%	3.4%	0%	
2	406	33	27	1		8.1%	6.7%	0.2%	
3	3654	796	457	50		21.8%	12.5%	1.4%	

Fig. 13. The effect of multiple markers on the ability to accurately discriminate between tumor tissue and non-malignant tissue.

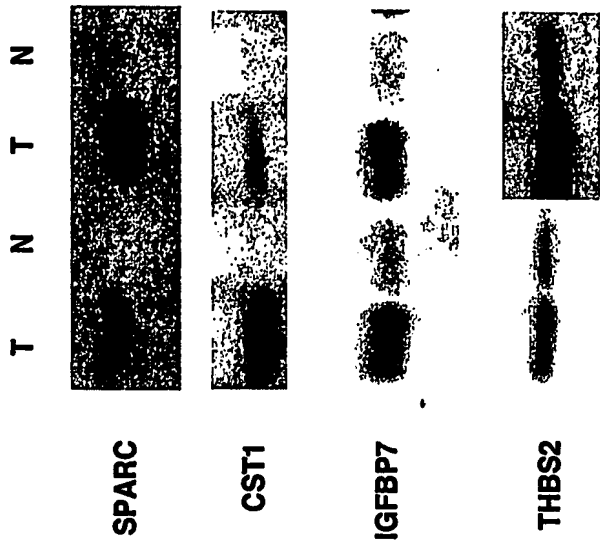


Fig. 14. Western analysis of markers in tumor and non-malignant tissue

Post Available Copy

marker tumor serum



Fig. 15. Western analysis of SPARC in gastric tumor material and serum.

Best Available Copy

104/104

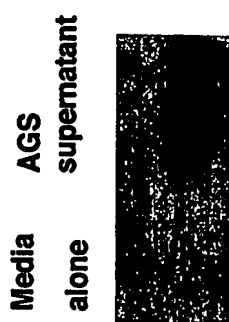


Fig. 16. Immunodetection of cystatin SN in the supernatant of the gastric cancer cell line, AGS.

Best Available Copy